THE PHYSIOLOGY OF THE AMINO ACIDS

Courtesy of Prof. L. B. Mendel

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FRANK P. UNDERHILL, Ph.D.

Professor of Pathological Chemistry, Yale University

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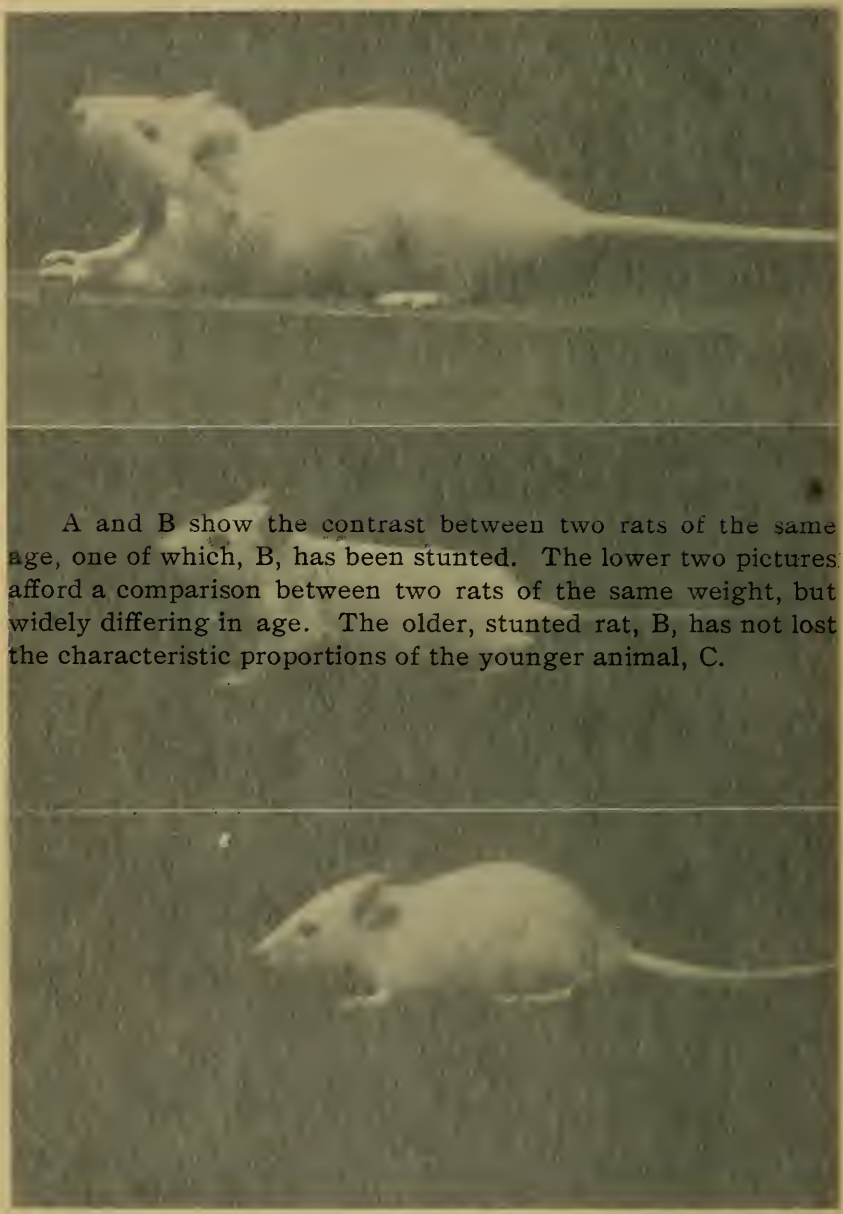

A and B show the contrast between two rats of the same age, one of which, B, has been stunted. The lower two pictures afford a comparison between two rats of the same weight, but widely differing in age. The older, stunted rat, B, has not lost the characteristic proportions of the younger animal, C.

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PREFACE

During the past few years the physiology of the amino acids has been subjected to much experimentation with the result that these protein cleavage products have assumed an ever increasing importance in the problems associated with nitrogenous metabolism. Owing largely to our too recent appreciation of the significance of these substances in metabolic processes there exists at present no compilation which furnishes an adequate conception of the rôles which may be played by the amino acids. It has been, therefore, the aim of the writer to gather together in one place the results which have thus far been obtained in the field of the biochemistry of the amino acids, thus affording the busy practitioner, and others whose resources for consulting original communications are limited, an opportunity of gaining a knowledge of the present-day problems in this field of nutrition. In the accomplishment of this purpose the writer has made no effort to include all the details or all the literature available upon a given topic, but has sought rather to indicate leading lines of thought. At the end of each chapter are given references in which all the important literature upon the topic discussed is cited.

It is assumed that the reader is familiar with the fundamental principles of metabolism, hence, in general, these have been omitted.

The author is deeply indebted to Professor Lafayette B. Mendel for suggestions, criticisms of the manuscript and for some of the plates presented.

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CHAPTER I

THE PROTEINS AND THEIR DERIVATIVES—THE AMINO ACIDS

The Proteins

The presence of nitrogen as a fundamental constituent of protoplasm attests the supreme importance of this element for the construction of living matter and the continued existence of organized life. It is well recognized, however, that all forms of nitrogen are not equally available for the maintenance of physiological rhythm. In support of this may be cited the fact that although the animal organism is continually surrounded by an atmosphere rich in nitrogen, little or none of this nitrogen can be utilized by the body for nutritional purposes. The organism possesses discriminating powers and demands nitrogen in a specific form, namely, such as that peculiar to protein and its derivatives. Protein material constitutes therefore an essential foodstuff and without it life would be impossible for any considerable period of time. "It is the chemical nucleus or pivot around which revolves a multitude of reactions characteristic of biological phenomena."

Viewed from the chemical standpoint protein is seen as a huge molecule, complex in structure, labile in character and therefore prone to chemical change. So large and intricate is the make-up of the molecule that chemists for generations have been baffled in their attempts to gain any adequate conception of its nature. At the present stage of our knowledge it is impossible to form any satisfactory definition of a protein based either on its chemical or physiological properties. In general, proteins contain about 15 to 19 per cent of nitrogen, 52 per cent of carbon, 7 per cent of hydrogen, 23 per cent of oxygen and 0.5-2.0 per cent of sulphur. Some also contain phosphorus or iron. They act like amphoteric electrolytes, that is, they are capable of forming salts with both acids and bases. Proteins belong to that class of substances known as colloids and as such do not possess the power to pass through animal or vegetable membranes. In a manner similar to colloids they may be separated from their solutions by suitable treatment with salts, such as sodium chloride, ammonium sulphate, etc. By a process known as "coagulation," which may be induced by the action of heat or the long continued influence of alcohol the proteins lose their colloidal characteristics which cannot be restored.

Many proteins are capable of crystallization and indeed may occur in nature in crystalline form. It has been found possible also to cause some to crystallize although their presence in nature as crystals is unknown. Some doubt has been cast upon the

probability of proteins, as we differentiate them at present, being chemical units, but since many of the crystalline plant proteins show a constancy of properties and ultimate composition there is little reason for the assumption that these at least are mixtures of two or more individuals.

Concerning the size of the protein molecule some idea may be gained when it is recalled that the molecular weight has been calculated to be approximately 15,000.

The proteins possess the property of turning the plane of polarized light to the left, the degree of rotation for an individual protein varying with the solvent employed.

Classification of Proteins

At present proteins are classified according to their physical properties, as, for example, their solubility in pure water, weak salt solutions and dilute acids and alkalies. It is well recognized that such a classification is far from ideal, but it is the most satisfactory plan that has been offered. When more complete knowledge is gained concerning the chemical make-up of the protein molecule a classification will undoubtedly be framed which will be based upon the presence or proportion of certain chemical groups in the different proteins.

All albuminous substances may be divided into

three large groups, namely, the *Simple Proteins,* the *Conjugated Proteins* and the *Derived Proteins. Simple Proteins* may be defined as substances which yield only α-amino acids or their derivatives on hydrolysis. *Conjugated Proteins* are substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt. As their name implies, the *Derived Proteins* are substances that have been formed from naturally occurring proteins.

The various sub-divisions of these large groups, as adopted by the American Physiological Society and the American Society of Biological Chemists, follow:

Simple Proteins	*Conjugated Proteins*
1. Albumins.	1. Nucleoproteins.
2. Globulins.	2. Glucoproteins.
3. Glutelins.	3. Phosphoproteins.
4. Alcohol-Soluble Proteins.	4. Hemoglobins.
5. Albuminoids.	5. Lecithoproteins.
6. Histones.	
7. Protamines.	

Derived Proteins

A. Primary Protein Derivatives.	B. Secondary Protein Derivatives.
1. Proteans.	1. Proteoses.
2. Metaproteins.	2. Peptones.
3. Coagulated Proteins.	3. Peptides.

Occurrence and Characteristics of Different Classes of Proteins

A. *Simple Proteins*

Albumins are simple proteins that are soluble in pure water and are coagulable by heat. *Globulins,* on the other hand, are insoluble in pure water but are readily soluble in dilute salt solutions. Albumins and globulins are generally found together in nature occurring, for example, in large quantity in the blood serum, white of egg, in the substance of cells in general, and in various seeds. Egg white may be divided into two parts by dialysis against distilled water—the globulin being precipitated owing to the diffusion of the salts from the solution which originally were present in quantity sufficient to hold the globulin in solution.

Glutelins are simple proteins insoluble in all neutral solvents but easily soluble in very dilute acids and alkalies. *Alcohol-Soluble Proteins* are simple proteins readily soluble in relatively strong alcohol (70 to 80 per cent), but are insoluble in water, absolute alcohol and other neutral solvents. These two groups of proteins occur together as constituents of the cereal grains. *Glutenin* and *Gliadin,* respectively, from wheat, are the best known examples of these two groups. They constitute the gluten of flour. The elasticity and strength of the gluten, and therefore the

baking qualities of a flour are influenced by the proportions of glutenin and gliadin.

Albuminoids may be defined as simple proteins which possess essentially the same chemical composition as the other proteins, but are characterized by great insolubility in all neutral solvents. Examples of this group may be found as the organic basis of bone (ossein), of tendon (collagen and its hydration product, gelatin), of ligament (elastin) and of nails, hairs, horns, hoofs, and feathers (keratins).

Histones are basic proteins which may be looked upon as standing between protamines and the more complex proteins. They are precipitated by other proteins and yield a coagulum on heating which is readily soluble in very dilute acids. The histones are soluble in water but insoluble in ammonia. They have been isolated from varied sources, as *globin* from hemoglobin, *scombron* from spermatozoa of the mackerel, *gaduhiston* from the codfish and *arbacin* from the sea-urchin.

Protomines are the simplest natural proteins. They are soluble in water, are not coagulable by heat, have the property of precipitating other proteins from their solutions, are strongly basic and form stable salts with strong mineral acids. Examples of protamines are *salmin* (from the spermatozoa of the salmon), *sturin* (from the sturgeon), *clupein* (from the herring), and *scombin* (from the mackerel).

B. *Conjugated Proteins*

Nucleoproteins are compounds of one or more protein molecules united with nucleic acid. The nucleoproteins, as their name implies, are the proteins of cell nuclei and give to the latter their character. The nucleoproteins are therefore found in largest quantity wherever cellular material is abundant, as in glandular tissues and organs. By artificial hydrolysis or during treatment in the alimentary tract a nucleoprotein is decomposed into protein and nucleic acid. Nucleic acid, of which there are several types, may be made to yield a series of well-defined compounds, the purine bases (xanthine, hypoxanthine, adenine and guanine), the pyrimidine bases (uracile, cytosine and thymine), a carbohydrate group (pentose or hexose) and phosphoric acid.

Glucoproteins are compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Particularly rich in glucoproteins are the mucus-yielding portions of tissues. They serve also as a cement substance in holding together the fibers in tendons and ligaments. An amino-sugar, glucosamine, has been isolated from some of the glucoproteins and it is generally regarded as constituting the carbohydrate radicle of these conjugated proteins.

Phosphoproteins are compounds of the protein molecule with some, as yet undefined, phosphorus-containing, group other than a nucleic acid or lecithin.

Conspicuous foods containing phosphoproteins are milk with its caseinogen and egg yolk with its vitellin. A trace of iron is also evident in these proteins and although it is possibly present as an impurity there is no evidence that it does not exist in combination with the protein.

Hemoglobins are compounds of the protein molecule with hematin or some similar substance. The coloring matter of the blood is hemoglobin which acts as oxygen carrier for the tissues and is characterized by holding iron as a constituent part in organic combination. Globin is the protein portion of hemoglobin. In certain of the lower animal forms copper enters into combination with protein forming hæmocyanin imparting a blue color to the blood.

Lecithoproteins are compounds of the protein molecule with lecithins. Lecithins are complexes characterized by yielding glycerol, phosphoric acid, fatty acid radicles, and a nitrogenous base, choline. The lecithins are present in all plant and animal cells but are especially abundant in the nervous tissues. They belong to the group of essential cell constituents.

C. *Derived Proteins*

Certain of the native soluble proteins upon continued contact with water, or the influence of enzymes or acid change their character and become insoluble. Such insoluble substances are called *proteans*. After repeated reprecipitation globulins may become insolu-

ble, that is, they are changed to proteans, and it is believed by some protein investigators that nearly every protein may assume a protean state.

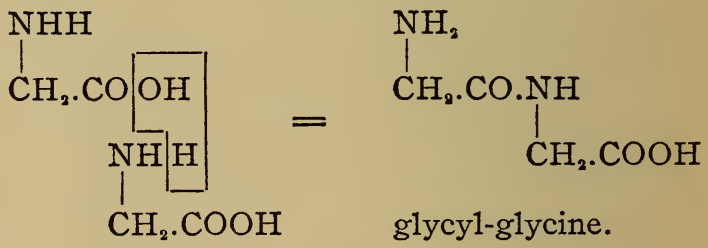
The *metaproteins* may be formed from simple protein by the action of acids and alkalies. In this instance, however, the change is undoubtedly more profound than in the case of the proteans. Formerly, metaproteins were termed albuminates, that formed by acid being called acid albuminate, that from the action of alkali being designated alkali albuminate. These substances are insoluble in neutral fluids but are readily soluble in an excess of acid or alkali. The metaproteins are of interest when it is recalled that the acid metaprotein arises as the first step in gastric digestion of protein and that likewise alkali metaprotein may be formed during pancreatic digestion.

The *coagulated proteins* can be produced from simple proteins by the long continued action of alcohol, stirring or shaking of their solutions, or by the influence of heat. In one instance, namely, the transformation of fibrinogen into fibrin in shed blood, the process has long been assumed to be induced by an enzyme. More recent work, however, tends to show that enzyme action is not concerned in the reaction.

The class of derived proteins called *Secondary Protein Derivatives* represent a more profound change from simple proteins than is true for the proteans, metaproteins and coagulated proteins which are grouped together as *Primary Protein Derivatives*. Of the secondary protein derivatives the *proteoses*

and *peptones* are characterized chiefly by their greater solubility and by the fact that, unlike most other proteins, they are diffusible through suitable membranes. They represent stages in gastric, pancreatic and bacterial digestions of protein and the peptones are regarded as products of greater cleavage than the proteoses. There are several proteoses, as *protoproteose, heteroproteose* and *deuteroproteose* and probably there may be several types of peptones. The proteoses are distinguished from the peptones principally in being precipitated from solutions by saturation with ammonium or zinc sulphate.

The *peptides* are "definitely characterized combinations of two or more amino acids, the carboxyl (COOH) group of one being united with the amino (NH_2) group of the other with the elimination of a molecule of water." For example, if two molecules of glycocoll (glycine)—amino-acetic acid—are condensed, a peptide, glycyl-glycine, will result. Thus—



The peptides are designated di-tri-tetra-peptides, etc., according to the number of amino acids in combination. The name polypeptides is also applied to these

substances. It is usually accepted at the present time that the peptones are relatively simple polypeptides, the line of demarcation between a simple peptone and a complex peptide not being well defined.

The Amino Acids

For nearly a century chemists have been seeking to establish the composition and structure of the protein molecule. Progress, which was slow and irregular in the earlier decades of this period, has taken rapid strides in the last twenty years, more intimate knowledge of the problem being gained during this interval than in all previous time. The investigation has been pursued in three directions—first the demolition of the molecule and the subsequent identification of the resulting fragments; second, the determination of the quantitative relationships of these fragments; and finally, attempts to unite the disintegration products in such a manner as to reproduce the original molecule.

After a considerable period of investigation it was established that, although the protein molecule may yield different types of substances according to the character of the means employed for disrupting it thus indicating a variety of possible lines of cleavage, hydrolysis furnishes the most promising types of units. Latterly, this type of chemical reaction has been employed exclusively and it has yielded the important information now available concerning the nature of the protein decomposition products. Each

protein investigated by this method was found to yield relatively large molecules, such as proteoses and peptones, and on further disintegration a series of comparatively simple nitrogenous substances of low molecular weight which belong to a definite group of chemical compounds—namely, the amino acids. An amino acid may be regarded as an organic acid in which one hydrogen is replaced by the amino group (NH_2), or viewed from another standpoint, an amino acid may be considered as a substituted ammonia, one hydrogen of ammonia, NH_3, being replaced by an organic acid. A description of the amino acids yielded by proteins follows.

Glycocoll or *glycine, amino-acetic acid.* $CH_2 < \begin{matrix} NH_2 \\ COOH \end{matrix}$ is the simplest of the products obtained from protein by hydrolytic cleavage and it was also the first to be discovered. Its separation dates back to 1820 in which year Braconnot obtained the substance by boiling gelatin with sulphuric acid, and because of its sweet taste called it sugar of gelatin. About twenty-five years later Dessaignes isolated it after a hydrolysis of hippuric acid. It was shown by Strecher in 1848 that glycocholic acid, then called cholic acid, consists of a combination of cholalic acid and glycocoll, and in consequence of its being a constituent of a bile acid, glycocoll assumed a position of some physiological importance. Its presence in various types of albuminoids, such as elastin, etc., was later demonstrated and finally it was shown to be a decomposition product of globulin. Glycocoll is not present in all proteins for albumin, casein, and hemoglobin fail to yield it, and from the vegetable proteins it is obtained in small quantities only. On the other hand, albuminoids are particularly rich in glycocoll. In an extract of the mollusc

Pecten irradians Chittenden found glycocoll in a free state; and it has been reported as occurring in the urine under various pathological conditions. After administration of benzoic acid to man and animals hippuric acid (benzoyl-glycocoll) is found in the urine—thus demonstrating a synthesis of hippuric acid from benzoic acid and glycocoll.

Alanine—α-amino-propionic acid. $CH_3.CH<\begin{matrix}NH_2\\COOH\end{matrix}$ was prepared synthetically previous to its isolation from among the protein decomposition products and was named by its discoverer, Strecher. Alanine has been shown to be a constant decomposition product of proteins.

Valine—α-amino-isovalerianic acid. $\begin{matrix}CH_3\\CH_3\end{matrix}>CH.CH<\begin{matrix}NH_2\\COOH\end{matrix}$

In 1856 v. Gorup-Besanez isolated a substance having the formula $C_5H_{11}NO_2$ from pancreas and because it possessed properties similar to leucine he looked upon it as a homologue of leucine and called it butalanine. Although a similar substance was isolated from certain seedlings by Schulze and Barbieri, and from the protamine, clupeine, by Kossel, it was not until 1906 that its identity was established by Fischer who gave it the name of valine. Valine is obtained from most proteins.

Leucine. α-amino-isobutylacetic acid.

$$\begin{matrix}CH_3\\CH_3\end{matrix}>CH.CH_2.CH<\begin{matrix}NH_2\\COOH\end{matrix}$$

Leucine was described by Proust in 1818 and was called oxide-caséux. Braconnot in 1820 obtained a substance from a hydrolysis of meat which on account of its glistening white appearance he called leucine. Liebig regarded it as one of the constituents of the protein molecule and this was later proved to be correct. Leucine is also a constituent of many organs and tissues occurring in the free state. It is yielded by both

animal and vegetable proteins and with the possible exception of arginine is the most widely distributed amino acid found as a protein cleavage product. Leucine has been found also in the urine under pathological conditions.

Isoleucine. α-amino-β-ethyl-propionic acid.

$$\begin{matrix} CH_3 \\ C_2H_5 \end{matrix}\!\!>\!CH.CH\!<\!\!\begin{matrix} NH_2 \\ COOH \end{matrix}$$

This amino acid was not described as a protein constituent until 1903 when it was isolated as a decomposition product of fibrin and other proteins by F. Ehrlich.

Norleucine. α-amino-normal-caproic acid. $CH_3.CH_2.CH_2.CH_2.CH.NH_2.COOH$. From the leucine fraction of the decomposition of the proteins of nervous tissue this amino acid has recently been isolated by Abderhalden and Weil. It is probable that other proteins may yield it also.

Phenylalanine. β-phenyl-α-amino-propionic acid.

$$C_6H_5.CH_2.CH\!<\!\!\begin{matrix} NH_2 \\ COOH \end{matrix}$$

Although it had been recognized for many years that a substance having the composition of $C_9H_{11}NO_2$ could be obtained by cleavage of both animal and vegetable proteins, it was Fischer who first proved the presence of phenylalanine as a protein derivative. In those proteins lacking tyrosine, as gelatin, for example, the aromatic ring is supplied by phenylalanine.

Tyrosine. β-para-oxyphenyl-α-amino-propionic acid.

$$HO.C_6H_5.CH_2.CH\!<\!\!\begin{matrix} NH_2 \\ COOH \end{matrix}$$

In 1846 Liebig isolated from a decomposition of cheese a substance possessing the property of crystallizing in silky needles. He named it tyrosine. Since then tyrosine has been regarded as a protein cleavage product. It was not until 1882,

however, that the structure of tyrosine was positively determined. Tyrosine is absent from the gelatine molecule. In acute yellow atrophy of the liver and in phosphorus poisoning it is claimed that tyrosine may be present as a urinary constituent.

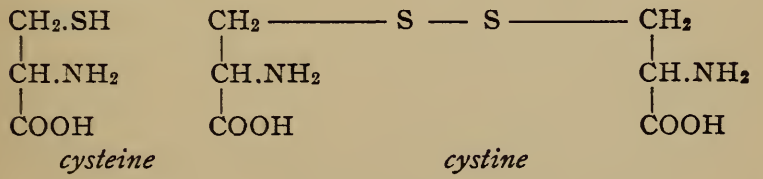
Serine β-hydroxy-α-amino-propionic acid.

$$CH_2(OH).CH<\begin{matrix}NH_2\\COOH\end{matrix}$$

Cramer found serine among the decomposition products of sericin (silk gelatin), and it was not obtained again until 1902 when Fischer isolated it from various proteins as a result of hydrolysis. He also definitely established its structure.

Cystine. di-cysteine or di-β-thio-α-amino-propionic acid. $HOOC.CH.NH_2.CH_2.S—S.CH_2.CH.NH_2.COOH$.

Cystine has been known since 1810 having been first described by Wollaston who separated it from a urinary calculus and called it cystic oxide. From that period, although cystine was repeatedly isolated from various organs of the body, as the liver and kidney, its presence as a regular decomposition product of protein was not established until 1899 when K. A. H. Mörner obtained it by a hydrolysis of horn. Baumann demonstrated the relationship of cysteine to cystine and thus revealed the structure of cystine. Cysteine and cystine bear the same relation to one another as does a mercaptan to a disulphide, thus,



cysteine *cystine*

Cystine is of considerable importance in metabolism inasmuch as it is the only known sulphur-containing amino acid in the protein molecule.

Aspartic Acid—Amino-succinic acid.

$$\begin{array}{l} CH_2.COOH \\ | \\ CH.NH_2.COOH \end{array}$$

Asparagine, the amide of aspartic acid, has been known since 1806, having been isolated from asparagus juice by Robiquet and Vanquelin. Upon boiling asparagine with lead hydroxide Plisson in 1827 obtained aspartic acid. In 1868 aspartic acid was shown by Ritthausen to be present as a product of hydrolytic cleavage of vegetable proteins. In a similar manner Kreussler obtained it upon hydrolysis of animal proteins and in 1874 it was isolated by Radziejeioski and Salkowski from a tryptic digestion of protein. Its structure was established in 1887.

Glutamic Acid (Glutaminic Acid) α-amino-glutaric acid.

$$\begin{array}{l} CH_2 \\ | \\ CH_2.COOH \\ | \\ CH.NH_2.COOH \end{array}$$

Although glutamic acid was first separated from a hydrolysis of wheat gluten in 1866 by Ritthausen its structure was not shown until 1890. Ritthausen demonstrated that it was an amino acid and from this fact together with its origin from gluten gave it the name of glutaminic acid. Glutamic acid was later shown to arise from hydrolytic cleavage of proteins of animal origin as well as from those derived from the vegetable kingdom.

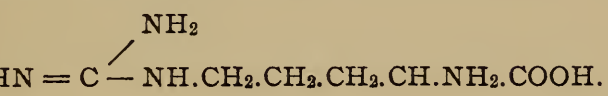
Lysine. α-, ε,-diamino-caproic acid.

$$H_2N.CH_2.CH_2.CH_2.CH_2.CH.NH_2.COOH.$$

Lysine is widely distributed as a protein constituent. It was first isolated from casein by Drechsel in 1889. Ellinger

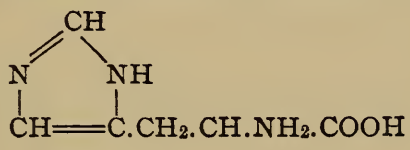
first demonstrated its structure in 1900 by obtaining cadaverine from it by putrefaction.

Arginine. α-amino-δ-guanidine-valerianic acid.



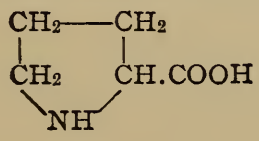
Among the products of a decomposition of casein Drechsel found a substance which he called *lysatinine.* Later, in 1894, Hedin demonstrated that this product was in reality a mixture of lysine and arginine. Arginine had been obtained previously by E. Schulze and Steiger from the seedlings of various plants. Urea and ornithine are among its decomposition products.

Histidine. β-imidazole-α-amino-propionic acid.



Histidine was discovered by Kossel in 1896 among the decomposition products of the protamine of sturgeon testes. From the fact that histidine, arginine, and lysine each contain six carbon atoms Kossel called these three substances the *hexone bases,* and they were regarded as a very important portion of the protein molecule. It was not until 1904 when the structure of histidine was shown by Pauly and Windhaus and Knopp that it was recognized to belong to a group of compounds entirely different from that including arginine and lysine.

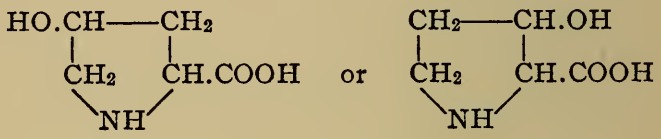
Proline. α-pyrrolidine-carboxylic acid.



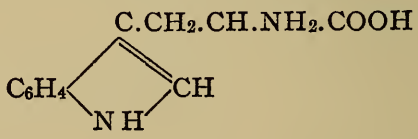
Proline was first isolated by Fischer from casein. Its presence in various other proteins was soon shown.

Oxyproline.

This amino acid was prepared from gelatin in 1902 by Fischer. Its structure is not yet definitely established although it undoubtedly possesses one of the following formulas.



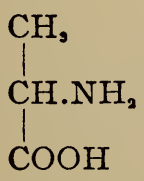
Tryptophane. β-indole-α-amino-propionic acid.



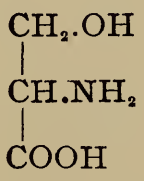
It was shown in 1826 by Tiedemann and Gmelin that when chlorine or bromine water is added to a tryptic digestion mixture a violet color is produced. Stadelmann named the substance giving this reaction proteinochromogen and Neumeister proved that any severe treatment of protein would cause the production of this compound to which he gave the name tryptophane. Hopkins and Cole in 1902 isolated from a tryptic digestion of casein a substance which gave all the reactions of tryptophane, namely, the violet coloration with bromine or chlorine, the Adamkiewicz reaction, and the production of indole and skatole as a result of putrefaction. In this manner the origin of the substances characteristic of putrefaction was made clear. The structure of tryptophane was regarded by Nencki as indole amino acetic acid. Ellinger, however, showed it to be an indole amino propionic acid.

Caseinic Acid, or diamino-trioxy-dodecanic acid. This compound has been isolated by Skraup from casein only. Its structure is still unknown.

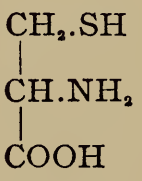
On inspection of these formulas it may be established that certain of the amino acids are very closely related; thus, glycocoll, the simplest of all, by introduction of the group (CH_3) becomes alanine. This substance possesses interest because several of the amino acids may be regarded as alanine derivatives. By the replacement of an (OH) group alanine becomes serine, or by substitution of an (SH) group alanine is changed to cysteine. If the phenyl group (C_6H_5) is introduced phenylalanine is obtained, and the additional substitution of an (OH) group leads to tyrosine.



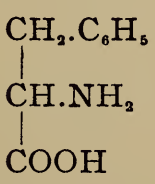
Alanine



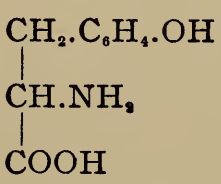
Serine



Cysteine

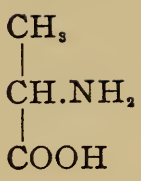


Phenylalanine

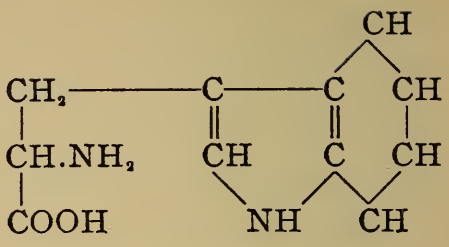


Tyrosine

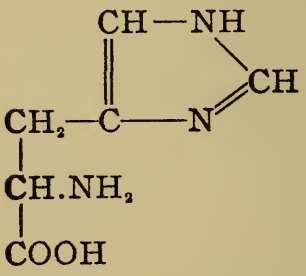
The introduction of the indole or iminazole group leads to the formation of tryptophane or histidine respectively.



Alanine

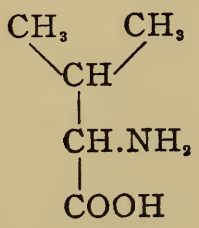


Tryptophane

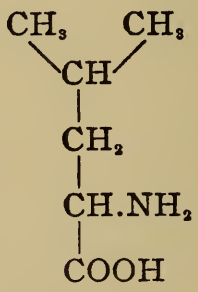


Histidine

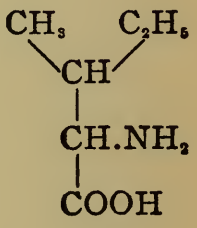
Again valine, leucine and isoleucine are closely related structurally as may be seen from the formulas following.



Valine



Leucine



Isoleucine

Viewed from another standpoint the amino acids may be divided into mono-amino acids,—glycocoll, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, serine, aspartic acid, and glutamic acid,—each containing, as the name implies a single amino (NH_2) group—diamino acids, containing two amino groups, as arginine, and lysine and finally the heterocyclic compounds as histidine, proline, oxyproline, and tryptophane.

The Quantitative Relationships of Amino Acids in Proteins

The most serious obstacle to the quantitative estimation of amino acids in hydrolysis mixtures has been that of inadequate methods of separation. By means of the ester method of E. Fischer this difficulty has been obviated in large measure. In Table I below are presented figures showing the yield of individual amino acids obtained by various investigators from representative simple proteins. The figures have not all been derived from use of the most exact methods of isolation, hence it is probable that they may not represent maximal values or be strictly correct. Nevertheless, they are sufficiently suggestive to demonstrate the distinct differences that exist between the simple proteins.

Table II undoubtedly gives the most accurate figures obtainable at present for the quantitative yield of

Table I.

Table Showing the Quantities of Amino Acids Obtained from Hydrolysis of Representative Simple Proteins and Common Foodstuffs

	Protamine (1) (4) *Salmine*	*Histone* (1) Globin from hemoglobin (Horse Blood)	*Albumin* (1) (5) Serum Albumin	Globulin (1) (5) Serum Globulin	Globulin (2) Crystallized Excelsin from Brazil Nut	Glutelin (2) Amandin from Almond	Alcohol Soluble Proteins Gliadin (2) (Wht)	Zein (2) (Maize)	*Phosphoprotein* Casein (1) Cow's Milk	*Albuminoid* Gelatin (3)	Egg (2) *Albumin*	*Beef* (2)	*Chicken* (2)	*Halibut* (2)	*Scallop* (2)
Glycocoll	.....	.....	0	3.5	0.6	0.51	0.02	.....	0	16.5	0	2.06	0.68	0	0
Alanine	.....	4.19	2.7	2.2	2.3	1.40	2.00	2.23	0.9	0.8	2.22	3.72	2.28	.....	.....
Valine	4.3	.....	.....	+	1.5	0.16	0.21	0.29	1.0	1.0	2.50	0.81	.....	0.79	.....
Leucine	.....	29.04	20.0	18.7	8.7	4.45	5.61	18.60	10.5	2.1	10.71	11.65	11.19	10.33	8.78
Isoleucine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Phenylalanine	.....	4.24	3.1	3.8	3.5	2.53	2.35	4.87	3.2	0.4	5.07	3.15	3.53	3.04	4.90
Tyrosine	.....	1.33	2.1	2.5	3.1	1.12	1.20	3.55	4.5	0	1.77	2.20	2.16	2.39	1.95
Serine	7.8	0.56	0.6	.....	.....	.....	0.13	0.57	0.23	0.4	.....	.....	.....	.....	.....
Cystine	.....	0.31	2.5	0.7	.....	.....	0.45	.....	0.06	.....	.....	.....	.....	.....	.....
Proline	11.0	2.34	1.0	2.8	3.6	2.44	7.06	6.53	3.1	5.2	3.54	5.82	4.74	3.17	2.28
Oxyproline	.....	1.04	.....	.....	.....	.....	.....	.....	0.25	3.0	.....	.....	.....	.....	.....
Aspartic Acid	.....	4.43	3.1	2.5	3.8	5.42	0.58	1.41	1.2	0.6	2.20	4.51	3.21	2.73	3.47
Glutamic Acid	.....	1.73	7.7	8.5	12.9	23.14	37.33	18.28	11.0	0.9	9.10	15.49	16.48	10.13	14.88
Tryptophane	.....	+	+	+	+	+	+	0	1.5	0	+	+	+	+	+
Arginine	87.4	5.42	.....	.....	16.1	11.85	3.16	1.16	4.84	7.6	4.91	7.47	6.50	6.34	7.38
Lysine	0	4.28	.....	.....	1.6	0.70	0	0	5.80	2.8	3.76	7.59	7.24	7.45	5.77
Histidine	0	10.96	.....	.....	1.5	1.58	0.61	0.43	2.59	0.4	1.71	1.76	2.47	2.55	2.02
Diaminotrioxydodecanic Acid	.....	.....	.....	.....	.....	.....	.....	.....	0.75	.....	.....	.....	.....	.....	.....
Ammonia	.....	.....	.....	.....	1.8	3.70	5.11	3.61	1.6	0.4	1.34	1.07	1.67	1.33	1.08
Total	110.5	69.87	42.8	45.2	61.0	59.00	65.82	61.53	53.42	42.1	48.83	67.30	62.15	50.25	52.51

(1) Abderhalden and Associates
2) Osborne and Associates
3) Fischer and Associates
(4) Kossel and Associa es
(5) Mörner

amino acids obtained by hydrolysis from proteins representing various groups of these substances.

TABLE II.

QUANTITATIVE COMPARISON OF AMINO ACIDS OBTAINED BY HYDROLYSIS FROM PROTEINS

(Compiled by T. B. Osborne, 1914)* (After Mendel)

	Casein	Oval-bumin	Gliadin	Zein	Edestin	Legumin (Pea)
Glycocoll	0.00	0.00	0.00	0.00	3.80	0.38
Alanine	1.50	2.22	2.00	13.39	3.60	2.08
Valine	7.20	2.50	3.34	1.88	6.20	?
Leucine	9.35	10.71	6.62	19.55	14.50	8.00
Proline	6.70	3.56	13.22	9.04	4.10	3.22
Oxyproline	0.23	?	?	?	?	?
Phenylalanine	3.20	5.07	2.35	6.55	3.09	3.75
Glutaminic acid	15.55	9.10	43.66	26.17	18.74	13.80
Aspartic acid	1.39	2.20	0.58	1.71	4.50	5.30
Serine	0.50	?	0.13	1.02	0.33	0.53
Tyrosine	4.50	1.77	1.61	3.55	2.13	1.55
Cystine	?	?	0.45	?	1.00	?
Histidine	2.50	1.71	1.49	0.82	2.19	2.42
Arginine	3.81	4.91	2.91	1.55	14.17	10.12
Lysine	5.95	3.76	0.15	0.00	1.65	4.29
Tryptophane, about	1.50	present	1.00	0.00	present	present
Ammonia	1.61	1.34	5.22	3.64	2.28	1.99
	65.49	48.85	84.73	88.87	82.28	57.43

*These analyses are combinations of what appear to be the best determinations of various chemists.

It may be seen from these tables that certain proteins, as serum albumin and casein contain no glycocoll, whereas serum globulin contains a small amount and gelatin a large quantity. Alanine presents variable figures but is usually present. The same may be said of leucine, phenylalanine, proline, and aspartic acid. Tyrosine may be absent as in gelatin. Glutamic

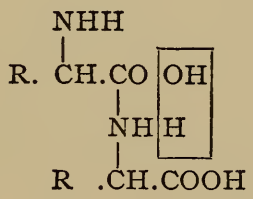
acid may show very wide variations being present to the extent of nearly 44 per cent in wheat gliadin whereas gelatin contains less than 1 per cent. Tryptophane may be absent as in zein and gelatin. Arginine shows great variation being present in largest quantity in the protamines (salmine). On the other hand, lysine is absent in salmine as well as in zein. In the protamine, salmine, histidine is not present but may be isolated from all other examples of simple proteins shown here. It is clear that in general the various proteins are made up of the same units and it undoubtedly follows that the individual protein characteristics are bestowed by the relative proportion of the units or by their absence.

In the tables given it will be observed that in most instances the total amino acids fall far short of the theoretical yield, a deficit of 40 to 50 per cent being in order. Previously it has been assumed that only a portion of the amino acids was known. At present, however, it seems very probable that the deficit is to be explained on the hypothesis of inadequate methods of analysis.

Synthetic Proof of the Structure of Protein

Since the time of Liebig it has been assumed that the protein molecule consisted of a huge complex of amino acids linked together in some unknown manner. There are many possibilities for such combinations and certain of them have been subjected to experimen-

tation without, however, yielding any very far-reaching conclusions. It remained for Emil Fischer and his associates in 1901 to conceive of a combination which undoubtedly will ultimately lead to a clear understanding of the structure of the protein molecule. These combinations of amino acids were termed polypeptides. Just as we have mono-, di-, or trisaccharides so there may be di-, tri-, etc.,-peptides. According to Fischer's method the amino acids are linked together by dehydration of their hydroxyl and amino groups, the carboxyl group of each acid being condensed with the amino group of its neighbor in the molecule, thus



By continued union of amino acids infinite possibilities of complexes are presented. Actually compounds containing as many as eighteen amino acids have been synthesized by Fischer and some of the products obtained have shown properties similar to those of the native protein.

After demonstration of the possibility of forming protein-like compounds by synthesis Fischer next attempted to determine whether similar simple complexes could be derived from proteins by suitable treat-

ment. For this purpose he employed a mild hydrolysis which only partially broke up the large aggregates formed and he succeeded in isolating from the products peptides identical with those made synthetically. Since then other investigators have separated similar compounds. One of the best proofs that proteins are built up of these amino acid complexes is that, also furnished by Fischer, of the action of various enzymes upon the synthetical products. It was found that with the exception of pepsin the various enzymes of the body are quite capable of hydrolyzing the polypeptides into amino acids.

Although these investigations prove beyond doubt that amino acids are linked together in protein in the form of polypeptides, there are possibilities of other forms of combination which will be revealed only by future research. For the present we are justified in accepting the hypothesis of the protein molecule as a huge complex polypeptide.

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CHAPTER II

DIGESTION, AND BACTERIAL ACTIVITY IN RELATION TO THE AMINO ACIDS

Concerning the nature of protein digestion Schaefer in 1898 wrote: "The products found toward the end of a proteid digestion *in vitro* are distinguished from the proteids from which they originate by being slightly diffusible. To this fact great importance was at one time attributed, because it was thought that only proteids in a diffusible form were capable of absorption, and hence that peptonization was in all cases a necessary preliminary. It is now generally admitted that many forms of native proteid are capable of entering the epithelial cells (of the intestine) without previous change by digestion or otherwise; and in those cases in which a proteid is incapable of direct absorption a much less profound change than peptonization is sufficient to render it so, namely, conversion into acid or alkali albumin." With regard to the extent of amino acid formation in digestion Schaefer says: "It is not known with certainty to what extent amino acids are formed from proteids, in the natural course of intestinal digestion. The experimental evidence is somewhat conflicting, but the majority of observers are of the opinion that but little proteid is absorbed as leucine or tyrosine, being nearly all absorbed as albumose or

peptone, or even at a still earlier stage. The only positive evidence as to the formation of leucine and tyrosine in natural digestion, rests on the amounts found in the intestinal contents during protein digestion." It is then stated that in general the quantities of amino acids present during digestion are small.

In the few years since the above was written the advances made in the chemistry of the proteins and of digestion have made necessary a radical revision of our ideas of the nature and extent of the alimentary treatment of protein. No longer tenable is the view that digestion stops with the transformation of insoluble and non-diffusible substances into compounds soluble and diffusible, nor can the idea be accepted of a distinction between directly assimilable and non-assimilable proteins. The change to "peptone" is now held to be merely an intermediate stage in digestion, not the end, as was once assumed. According to the latest conception of protein digestion a profound disintegration occurs, the ultimate products formed being a variety of polypeptides and amino acids. Digestion, in accordance with this idea, consists in a series of hydrolytic cleavages which are induced through the agencies of the enzymes present in the gastro-enteric tract. The products formed by these enzymes undoubtedly are identical with those produced outside the body by means of the action of acids. Amino acids therefore must be looked upon as the ultimate nitrogenous foodstuffs—it is to these substances that the organism must look for its essential requirement of nitrogen.

Are Amino Acids Formed during Gastric Digestion of Protein?

Protein digestion is initiated in the stomach through the action of gastric juice—the active constituents being pepsin and hydrochloric acid. In investigating the nature and extent of gastric digestion three general methods have been employed—as follows: (1) the stomach tube method, the only procedure applicable to man, whereby the stomach contents are withdrawn at intervals after a meal, (2) animals fed definite diets are quickly killed at varying periods of time and the stomach contents examined; or animals are killed with the stomach empty, food introduced and analyzed at intervals, (3) the polyfistulous method—fistulas being inserted in the stomach and at various points in the intestine, the food products being withdrawn through these openings.

What products are formed in the stomach under the influence of peptic digestion? It is self-evident that experiments carried out under artificial conditions, as in beakers, can afford no positive assurance that the products are identical with those formed in the stomach. Kühne was the first to demonstrate that pepsin digestion *in vitro* leads only to the formation of proteoses and peptones. On the other hand, numerous recent investigations have shown by the polyfistulous method that under normal conditions also proteoses and peptones represent the final stages in gastric digestion of protein. All the protein does not of necessity reach

the peptone stage. Indeed, in general the process goes only as far as the proteose stage as may be seen from the following table from London. In these experiments dogs were fed different types of proteins and from a fistula below the pylorus the products were collected.

Kind of Protein Fed	*Percentage of Proteoses Found*
Egg Albumin	72.5
Gliadin	67.7
Edestin	60.3
Casein	59.1
Gelatin	50.6
Serum Albumin	46.1

London and his co-workers also found that upon feeding varying quantities of the same protein a definite proportion of proteoses was always formed, thus—

Quantity of Gliadin Fed in grams	*Percentage of Proteoses Found*
25	80.8
50	86.1
75	86.5
100	84.9

It is therefore probable that ingested protein enters the duodenum largely in the form of proteoses and to a smaller extent as peptones.

By long continued action of both artificial and normal gastric juice various investigators have observed the gradual formation of amino acids. These results obtained from digestive mixtures allowed to stand for months cannot be regarded as applicable to normal

stomach digestion which is at most a matter of hours. They may be explained in several ways, as for instance in those cases where extracts of the stomach were employed amino acids may arise from autolytic processes, or perhaps in all cases from the action of hydrochloric acid alone. Protein is a labile molecule which apparently needs slight inducement to start on the downward path to its demolition into amino acids. The formation of amino acids in gastric digestion under normal conditions seems hardly probable. Against such an idea may be set the fact that pepsin-hydrochloric acid is utterly incapable of breaking down artificial polypeptides thus far tested. On the other hand, they are readily split by pancreatic juice. It would seem therefore that in peptic digestion neither amino acids nor relatively simple polypeptides are normally found in significant amounts.

Gastric digestion, however, has the very important function of preparing protein for the later action of trypsin and the intestinal juices. Fischer and Abderhalden have shown that tryptic digestion is much more rapid and complete when protein has been previously acted upon by pepsin-hydrochloric acid. If casein is first digested with an artificial gastric juice and then subjected to the influence of trypsin amino acids like proline and phenylalanine could be isolated. Treated with trypsin alone casein failed to yield the free amino acids; instead a corresponding polypeptide was present.

One may conclude therefore that although gastric digestion fails to yield amino acids directly it aids in

their rapid formation indirectly by facilitating the action of trypsin.

Intestinal Digestion

Kühne made the important discovery that there is an essential difference between the digestive action of trypsin and that of pepsin. He stated that the influence of the former does not cease with the formation of peptone but is carried to a stage where crystalline products appear—the amino acids. As late as 1900, however, these substances were regarded as by-products in natural digestion—of little significance and formed in relatively small quantities. At that time the cleavage products recognized were leucine, tyrosine, aspartic acid, glutamic acid, lysine, arginine and histidine and proteinochromogen (see Chapter I). With the growth of knowledge concerning protein chemistry most of the characteristic amino acids have since been isolated from intestinal contents.

In 1906 Cohnheim gave a new meaning to intestinal digestion by his discovery of an enzyme capable of splitting proteoses and peptones into simpler products. Cohnheim was of the opinion that synthesis of protein from peptones occurred in the intestinal wall. While endeavoring to determine this point he noted that the characteristic peptone reaction disappeared. Its disappearance was not due to protein synthesis as was early assumed, but because crystalline decomposition products were formed from it. This chemical trans-

formation was shown to be enzymatic in nature and to the enzyme Cohnheim gave the name *erepsin*. Later investigators showed that erepsin is quite specific in its action—it has no influence upon native proteins with the exception of casein and gelatin—but is capable of completely transforming proteoses and peptones into amino acids, such as leucine, tyrosine, lysine, histidine, and arginine. In intestinal digestion, therefore, two agencies are to be considered in protein disintegration, namely, trypsin and erepsin. From these two different types of activity one may perhaps draw the conclusion that there is a purposeful function for each. It may be imagined for instance that trypsin may perform a twofold function, the degradation of the protein molecule which may have escaped gastric digestion to the proteose or peptone stage, or completely to amino acids. Erepsin on the other hand is present to guarantee that all complicated structures as proteoses, peptones, or polypeptides are reduced to their simplest terms. It is apparent, therefore, from the distribution of enzymes in the intestinal tract that there is a natural provision for ingested protein to be subjected to a series of hydrolytic cleavages whereby only relatively simple amino acids are finally present.

Although it was generally admitted that protein digestion may proceed to the stage of amino acids it was exceedingly difficult to prove the fact when applied to the alimentary tract under normal conditions. The difficulty was twofold in nature. In the first place, demolition of the protein molecule is not of the nature

of an explosion resulting in a large number of fragments scattered about, but instead it may be looked upon as a kind of slow erosion whereby certain projecting pieces are rubbed or broken off. Secondly, absorption takes place rapidly and the erosion products have a tendency to disappear from the alimentary canal. A knowledge of the thorough character of intestinal digestion has been made possible through the employment of the polyfistulous method devised by London. Animals with a series of fistulas along the intestinal tract were fed gliadin and from successive openings the enteric contents were examined for the quantity of tyrosine and glutamic acid present. It was shown that in the duodenal contents 0.75 gram tyrosin and 2.5 grams of glutamic acid were present, in the jejunum were 1.1 gram tyrosin and 20.9 grams of glutamic acid while the ileum yielded only a trace of tyrosin and 33 grams of glutamic acid. Similar experiments with casein and meat yielded comparable results. From these observations it is quite evident that the processes of digestion in the intestine are gradual in nature but the rate of disintegration is much greater than obtains in artificial digestion mixtures. The apparent explanation for the slower rate of hydrolysis in *in vitro* experiments is that the digestion enzymes form compounds with the amino acids split off and thus are rendered inactive. This inactivation probably does not occur to any extent in the intestine because the amino acids do not accumulate therein, undoubtedly being absorbed almost as soon as they are split off.

The small intestine, therefore, may be regarded as the seat of profound protein digestion, the products arising being the amino acids typical for hydrolytic cleavage of protein. Undoubtedly all digestible proteins are ultimately reduced to the condition of amino acids. From this it follows according to present views that nitrogenous metabolism is concerned mainly with the amino acids and the transformations which they undergo.

Intestinal Bacteria and the Amino Acids

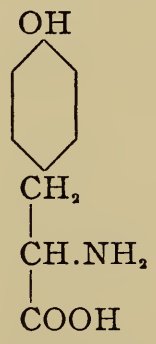
In the early days of the history of protein digestion great difficulty was experienced in the determination of the actual products formed because of the accompaniment of putrefaction. This was especially true for tryptic digestion where it is desirable to maintain an alkaline medium, an environment also favorable for bacterial growth. Kühne was the first to demonstrate the activity of trypsin in the presence of antiseptics and through the employment of antiseptic digestion mixtures a sharp division line was soon drawn between the products of tryptic digestion and those formed by bacterial agencies.

In general the products of putrefaction are identical whether formed outside the body or within. The type of action is similar to other kinds of digestion activity. Indeed, there is little doubt that the same kind of agencies are at work in the two instances, namely, enzymes. In the one case they are present in a secre-

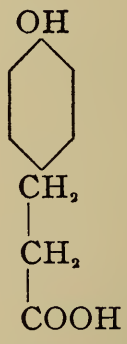
tion, as in intestinal juice, in the other instance they are contained within an organism. In bacterial digestion the first stages of digestion are very similar to those induced by trypsin. If the protein is insoluble solution is first effected which is not a rapid process as in the case of trypsin. The proteoses and peptones are next formed but are quickly transformed into lower decomposition products. Proteoses and peptones are much more readily attacked than are the native proteins, which may not begin to undergo a profound change until the former have been broken up to smaller molecules.

Putrefaction may be regarded as causing a different type of cleavage than occurs in ordinary tryptic or intestinal digestion as exemplified by the specific substances produced. It would appear much more likely, however, that the early stages of tryptic digestion and those induced by bacteria are identical in both instances, amino acids being the final products. On the other hand, little or no putrefaction occurs in the small intestine and there is little reason to assume that under ordinary circumstances any unchanged protein or perhaps even proteoses or peptones succeeds in passing the ileo-caecal valve. It is therefore probable that normally putrefactive bacteria act upon the amino acids rather than upon their precursors, the complex protein molecules. It is even doubted whether pure solutions of native proteins will putrefy directly. Accepting the hypothesis that it is the amino acids which are concerned primarily in putrefactive processes the forma-

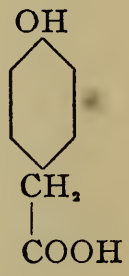
tion of the substances characteristic of putrefaction is readily understood. The amino acids which are especially susceptible to bacterial action are tyrosine and tryptophane. From tyrosine a whole series of compounds may be formed and are regularly present as putrefactive products, as for example, paroxyphenylpropionic acid (hydro-paracumaric acid), and paroxyphenylacetic acid (also phenylpropionic and phenylacetic acids), as well as paracresol and phenol. The relationships are readily seen from the following formulæ:



Tyrosine, or p. oxy-phenyl α-amino-propionic acid



p. oxy-phenyl propionic acid

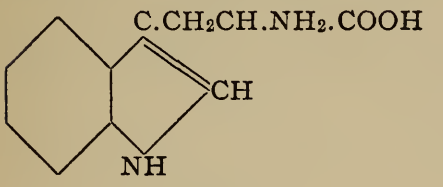


p. oxy-phenyl acetic acid

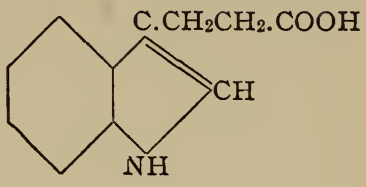
p. cresol

Phenol

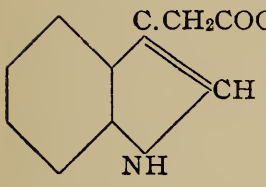
From tryptophane the malodorous bodies indole and skatole may be produced, thus:



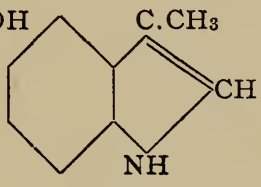
Indole-amino-propionic acid
Tryptophane



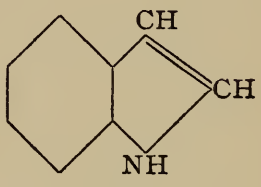
Indole-propionic acid



Indole-acetic acid



Skatole

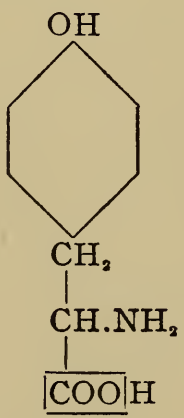


Indole

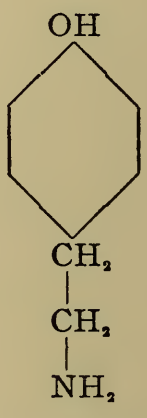
In the explanation of these changes in both instances it is seen that the types of chemical reactions are identical. First deamination or splitting off of ammonia, NH_3, occurs. This is followed by a cleavage of carbon dioxide, oxidation, and finally demethylation. The chemical transformations therefore are quite varied and extensive.

When putrefaction is mentioned one invariably thinks of indole, skatole, the oxy acids, etc. These compounds, however, by no means represent all of the substances actually formed for a type of chemical compound has been isolated which is also peculiarly

characteristic of putrefaction—namely, the amines. Dixon and Taylor in 1907 aroused considerable interest by the publication of their observation that alcoholic extracts of the human placenta when injected intravenously caused a marked rise in blood pressure and contractions of the pregnant uterus. It was later shown that these phenomena failed to appear in placental extracts free from putrefaction. Evidence was soon produced showing that putrefaction of the placenta caused the production from tyrosine of a new body, namely p-oxyphenylethylamine. This substance was isolated from a pancreas digestion several years previously by Emerson and its production as a product of tryptic action was regarded as unique. In the light of present knowledge there is little doubt that here also it was formed through bacterial agency. This new substance is produced by the liberation of CO_2 from tyrosine, thus:



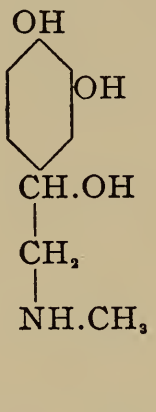
Tyrosine



p. oxyphenyl-ethylamine

To this compound has been given the name *tyramine*. It is of special significance both from the chemical and pharmacological standpoints because of its resemblance in both respects to epinephrine.

Tyramine



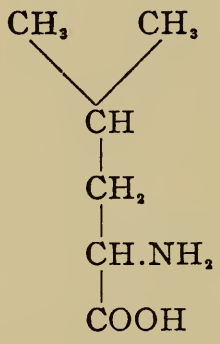
Epinephrine

Tyramine acts upon the sympathetic nervous system as does epinephrine. Its action, however, is somewhat weaker. Its effects are produced whether absorbed from subcutaneous tissues or from the alimentary canal. A further interest attaches to tyramine in that it is one of the substances that confers upon ergot its characteristic action on the uterus.

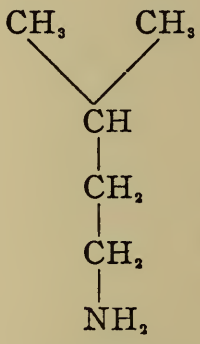
Not only is tyramine found in putrefaction mixtures without the body, but it has been isolated from the contents of the large intestine and it may, therefore, be looked upon as a product formed regularly in the body. On the other hand its presence in the alimentary canal does not necessarily imply that it was formed

there for it has been shown quite recently that it may be ingested with certain food products. Thus tyramine occurs in such varieties of cheeses as the Camembert, Roquefort, Emmenthal and even the American cheddar cheese is not free from it.

In a manner similar to the formation of tyramine we may have amines produced from other amino acids by bacteria. From leucine may be formed isoamylamine, thus:

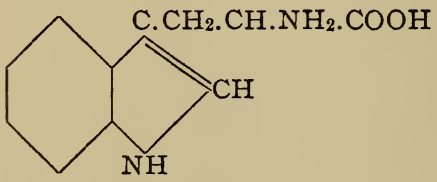


Leucine

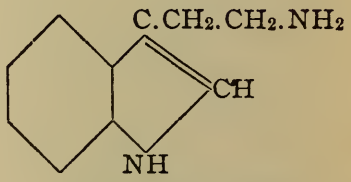


Isoamylamine

From tryptophane a corresponding amine may be produced, thus:



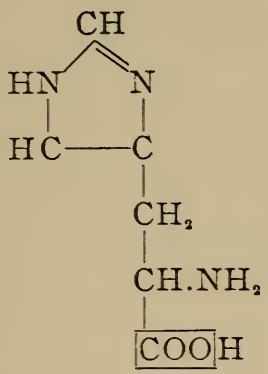
Tryptophane



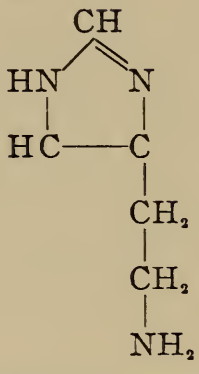
Indole-ethylamine

When histidine is subjected to the action of putrefactive bacteria it is transformed to β-iminazolylethyl-

amine or as it has been called *histamine*. The reaction occurring follows.



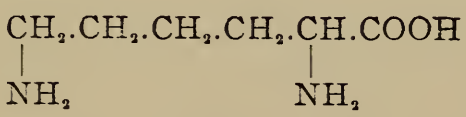
Histidine



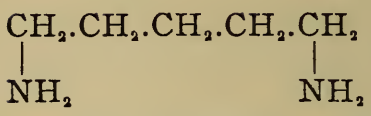
β-iminazolylethylamine

This substance besides possessing an action upon the nervous system is capable of producing symptoms identical with those of anaphylactic shock. Its presence in the alimentary canal has also been demonstrated.

The diamines, cadaverine and putrescine, arise in the alimentary through the action of bacteria. Cadaverine is produced in the following manner, lysine serving as the mother substance. Lysine, which has the following formula:

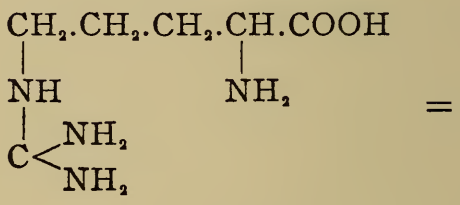


by cleavage of carbon dioxide yields cadaverine which has the structure below

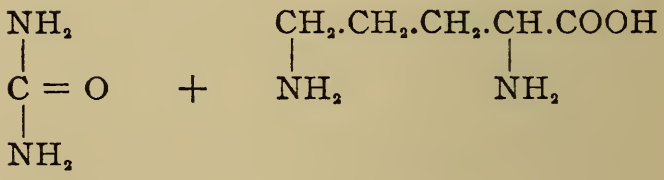


Cadaverine or Pentamethyldiamine

Arginine, another amino acid, is the mother substance of putrescine. Arginine under suitable conditions yields urea and ornithine, thus:

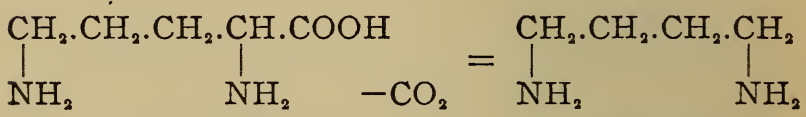


Arginine



Urea Ornithine

Ornithine by cleavage of carbon dioxide yields putrescine or tetramethyldiamine.



Ornithine Putrescine or Tetramethyldiamine

It is exceedingly probable that the purpose of protein digestion is the reduction of these complex molecules to the form of crystalline products, the amino acids. Putrefaction is also concerned with these substances forming from them compounds which may exert perhaps at times a more or less deleterious action as, for example, indole or skatole, but also transforming the amino acids into products, as tyramine or histamine, which time may show to have distinct physiological activities in keeping normal the adjustment of nutritional rhythm.

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CHAPTER III

THE ABSORPTION OF PROTEINS AND AMINO ACIDS

The views which have been held from time to time relative to the character of the protein material or products capable of absorption have been greatly influenced naturally by the ideas concerning the nature and extent of protein digestion prevalent at a particular period. It is obvious that in the days of Liebig and his contemporaries when digestion was assumed to be little more than a process whereby proteins were rendered soluble that the conception of the absorption of unchanged protein should hold sway. Later, after Kühne had added his contributions to the knowledge of digestion, theories of absorption were correspondingly modified. Since the extent of formation and significance of the amino acids have become better appreciated our present-day views as to absorption are likewise undergoing modification.

Absorption from the Stomach

A great deal of discussion has taken place regarding the question of gastric absorption of protein. It has been asserted by Tobler that as much as 22 to 30 per cent of the material in the stomach after a protein

meal is absorbed. London and his co-workers, on the other hand, deny that any absorption takes place. Abderhalden with Prym and London have apparently decided the question in the negative for they have demonstrated that amino acids fed to a dog with several fistulæ almost completely pass the pylorus, the first absorption occurring in the duodenum. Under normal conditions, therefore, gastric absorption so far as protein is concerned may be regarded perhaps as a negligible factor.

Looked at from another viewpoint, that of the present with its modified ideas of the purpose of digestion, one would naturally expect little or no absorption from the stomach. If the view is correct that the purpose of alimentary treatment of protein is to hydrolyze this substance to either a polypeptide or amino acid stage then it is reasonable to assume that these are the products absorbed, rather than the proteoses or peptones. Inasmuch as gastric digestion fails to decompose protein to the stage of products naturally absorbed it is reasonable to assume that the stomach is not an organ adapted for extensive absorption under ordinary circumstances. On the other hand, Folin and Lyman have shown conclusively that amino acids, Witte peptone and urea, may be absorbed from the stomach when a ligature is tied around the pyloric opening. In view of these conflicting facts one is hardly justified in making a positive statement as to the importance of the stomach in the absorption of nitrogenous decomposition products.

Intestinal Absorption

From extensive experimentation it would appear that the small intestine is capable of absorbing unchanged native proteins and their decomposition products the proteoses peptones and amino acids.

Absorption of Undigested Protein

It was pointed out by Voit and Bauer in 1869 that undigested proteins such as serum or natural egg albumin may be absorbed by the small intestine, an observation which has been repeatedly confirmed by others. It has been suggested that this is not the manner in which most of the absorption takes place, only enough protein being absorbed to replace worn-out tissue, the excess being oxidized without ever having entered into the tissue metabolism proper. Various explanations have been offered to account for the fact of absorption of undigested protein. The most obvious assumption to make is that enzymes must have been present in the intestine resulting in hydrolysis to amino acids and hence their absorption. This point, however, is not valid since the absorption was too rapid to admit the possibility of such an hypothesis. Again, it has been assumed that the experimental conditions rendered the intestinal wall unusually permeable, thus allowing protein to pass. It is possible that these results may later be explained on grounds other than that of absorption, for Abderhalden, Funk, and London after introducing excessive amounts of protein into the

intestine failed to obtain any reaction with the precipitin test. If protein were actually absorbed unchanged in its natural form it is almost incredible that the precipitin test failed to demonstrate its presence when the extreme delicacy of this reaction is recalled.

What Happens to Protein Parenterally Introduced?

If it is possible for unaltered native protein to be absorbed by the intestinal epithelium is it capable of supplying the nitrogenous needs of the body? Or what changes does it undergo after absorption? In attempts to answer these questions endeavors have been made to follow the fate of native proteins introduced into the organism with avoidance of the gastroenteric tract. For many years it has been accepted that protein introduced parenterally may be utilized in part at least. This view was initiated from the investigations of Zuntz and v. Mering and Neumeister. Since then it has been repeatedly confirmed by a long list of observers. Although it is generally admitted that parenterally introduced protein reappears in the urine only in small measure, there is not a unanimity of opinion as to its fate. Even though the intravenous injection of egg albumin fails to lead to a large output of protein in the urine it has been agreed that its failure to be eliminated by the kidney is no evidence of its utilization in the tissues. In such an argument one might assume with reason that the protein could be excreted through the bile, be poured into the intes-

tine, undergo intestinal digestion and eventually be absorbed in the form of protein decomposition products. Experiments to test this point have been carried out. It has been shown that when a solution of casein is introduced directly into the blood stream a small part may reappear in the bile

On the other hand, when egg white or serum are injected subcutaneously into dogs and goats a goodly portion of the protein may be eliminated in the form of non-coagulable protein. This observation would tend to demonstrate the non-utilization of the injected protein as such and points out that it undergoes a change in its transit through the organism. In the blood also a non-coagulable protein, perhaps a proteose, is detectable, and a marked increase in nitrogen of the urine—in the form of urea—is apparent. Indeed, nitrogen equilibrium may be maintained under these circumstances when animals are given a sufficiency of carbohydrates.

In most of the work on parenteral absorption of protein the material introduced did not possess enough differentiation from other body proteins to distinguish it from them. Borchardt conceived the idea of injecting a protein with peculiar properties and chose hemielastin; after intravenous injection this substance was present in the wall of the small intestine, and Borchardt concluded that the protein was either on its way for excretion by the gut or further changes by the intestinal juices to prepare it for utilization by the tissues, or finally had found its way into the intestine by way

of the bile. The last hypothesis did not appear likely however, since none of the introduced protein could be found in the liver.

From the foregoing arguments it is clear that the apparent utilization of parenterally introduced protein may be disposed of in at least three ways: 1. The direct utilization by the tissues, for which there is little or no evidence. 2. The excretion of the injected material into the intestine where it is subjected to the action of digestive enzymes, finding its way either directly into the intestine or indirectly through the bile. There is little doubt that a certain portion of the injected material is treated in this manner. 3. The transformation of the native protein in the tissues into smaller fractions such as proteoses, peptones or amino acids. Heilner has suggested that the utilization of parenterally introduced protein is induced by the generation of a specific enzyme capable of bringing about a hydrolysis.

In the last suggestion it is probable that we have the true explanation for the phenomenon under discussion for Abderhalden and his co-workers have demonstrated that the parenteral introduction of native protein or of peptone gives to the blood serum of the animal the power of decomposing these substances, and this power is destroyed by heating to 60° to 65° C. Over-feeding by mouth confers upon the blood serum the same property. The acquisition of this power on the part of the blood serum may be regarded, as Heilner suggested, as a generation of an enzyme or

it may be possible that the transport of the foreign material through the tissue has carried with it into the blood enzymes already existing in other parts of the organism. Be this as it may, it is very probable that protein retained in the body after parenteral introduction or even perhaps after absorption from the intestinal canal without profound disintegration eventually undergoes decomposition into simpler products after reaching the blood stream. It would appear from this statement, therefore, that the tissues must prefer, to say the least, their pabulum in the form of relatively simple compounds rather than as complex molecules like the native proteins.

Absorption of Proteoses and Peptones

It was early discovered that peptone left in contact with the living intestinal wall disappeared or at least failed to show its characteristic reactions. From these observations Hofmeister formulated the theory that the peptones were taken up by the leucocytes of the intestine after absorption and by them transformed into protein and distributed to the tissues. This hypothesis failed to meet with the approval of Heidenhain, who, although believing in the conversion of peptone to protein, assigned to the intestine itself the important rôle of this transformation. In confirmation of the correctness of this idea may be cited the experiments of Hofmeister and Neumeister who demonstrated that peptone introduced directly into the blood

was treated as so much waste material being eliminated by the kidneys. Moreover, he also failed to find any trace of peptone in the blood or lymph of animals at the height of digestion.

The experiments cited above failed to throw any light upon the fate of the peptone, beyond its disappearance. In later experiments Neumeister showed the presence of leucine and tyrosine in the intestine after introduction of peptone, thus indicating a further decomposition of the peptone. Even though it might be accepted that peptone placed in the intestine undergoes a further breakdown to amino acids there still existed no proof that the amino acids were absorbed as such. It is possible to assume a synthesis of the amino acids back into protein in the act of absorption through the intestinal wall. An example of such a reaction is found in the digestion of fat where fat is split into fatty acids or soap and glycerol and regenerated as fat during the process of absorption.

It was Cohnheim's attempt to isolate this hypothetical protein from the intestinal wall which led to his discovery of the enzyme erepsin already considered the action of which has had a tendency toward filling in some of the gaps in our conception of the nature of intestinal digestion and absorption. Although it had been recognized for many years that amino acids are formed in intestinal digestion they were regarded as by-products and quite unimportant. As a result of the discovery of erepsin the purpose of the formation of amino acids first received its due recognition.

That all of the older investigators did not regard the direct absorption of protein or even of such large molecules as peptone as essential for nutrition may be seen from the view formulated by Salkowski and Leube. According to this suggestion leucine may be regarded as a substance capable after absorption of being built up into protein and therefore leucine might be looked upon as a stage in protein regeneration. Against such a view, however, stood the fact that the amino acids were not at that time demonstrable in the blood or lymph.

From the numerous researches carried through concerning the absorption of proteoses and peptones from the intestines, the conclusion may be drawn that although these proteins disappear when placed in contact with the intestinal mucosa, there is no evidence of their absorption as such for they can be found neither in the blood nor in the lymph. On the other hand, inasmuch as their disappearance from the intestine is coincident with the appearance of amino acids, an enzyme being furnished which specifically effects such a transformation, it is probable that these proteins are absorbed only in the form of amino acids.

The Absorption of Amino Acids

As has been stated previously the presence of amino acids in the small intestine has long been known. Their absorption therefrom, however, has been a matter of conjecture. Inasmuch as their presence in the blood

or lymph could not be detected the theory of their synthesis to protein coincident with their absorption was promulgated. To Folin we owe the first indirect proof of the absorption of amino acids from the intestines. By a set of delicate methods adapted for the partition of different forms of nitrogen he has succeeded in demonstrating an appreciable increase in the "non-protein" portion of the blood, after introduction of amino acids into a loop of intestine. Although the amino acids themselves were not isolated the non-protein fraction of the blood was so significant as to leave no doubt of amino acid absorption. Van Slyke and Meyer strongly fortified the view by the use of a different method. The quantity of amino acids in the circulation at one time is so small as to have escaped detection by methods previously in use. It remained for Abderhalden to demonstrate the presence of amino acids in the blood under normal circumstances and actually to isolate them. This he accomplished by employing fifty liter lots of blood. From such large volumes he succeeded in separating and identifying proline, leucine, valine, aspartic acid, glutamic acid, alanine, glycocoll, arginine, histidine, and lysine. In no instance did he obtain more than 0.5 gram of any one substance. The absorption of amino acids as such is, therefore, an assured fact.

Absorption from the Large Intestine

Recalling to mind the heterogenous mixture of substances that may reach the large intestine one at once

realizes the great number of compounds that may find their way into the blood stream. Leaving out of consideration any residue of undigested protein we may confine our attention to the possibilities of proteose and peptones, the amino acids and the derivatives of the latter. The evidence of the absorption of proteose and peptone derivatives is decisive since Folin and Denis have demonstrated an increase in the non-protein nitrogen of the blood after placing Witte peptone in a ligatured loop of the large intestine. The absorption from the large intestine, however, is much slower than obtains in the small intestine. In a similar manner Folin and Denis have observed the absorption of different amino acids and urea. Throughout the entire intestinal canal, therefore, the absorption of amino acids may be regarded as a normal process.

The absorption of the well-known typical products of putrefaction needs only brief description since their fate has long been recognized. Absorption of indole, skatole, phenol, cresol, etc., is certain since their addition products are found in the urine. Thus indole is absorbed, carried to the liver through the portal vein, oxidized to hydroxyindole (indoxyl), combined with sulphuric acid and eventually is eliminated as the potassium salt, indican—its amount being indicative of the extent of intestinal putrefaction. Or indole may be combined in part after oxidation with glycuronic acid and be excreted as a glycuronate. Phenol and cresol may likewise be eliminated in the urine as ethereal sulphates.

The fate of the amines formed in putrefaction is also fairly well established, at least in certain instances. Thus, for example, it is known that the amine formed from tyrosine, p.oxyphenylethylamine in passing the organism is transformed to and excreted as p.oxyphenylacetic acid.

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CHAPTER IV

IN WHAT FORM DOES INGESTED PROTEIN ENTER THE CIRCULATION?

Our conception of the nature of metabolic processes in the tissues will be more or less modified by the view accepted concerning the degree and character of disintegration of protein induced in the alimentary tract and the form of the products absorbed. This being so a consideration of the hypotheses that have been advanced relative to the fate of protein after its disappearance from the gastro-enteric tract is now in order. This fate of ingested protein has been explained in at least four different ways, namely:

1. The proteins are absorbed with little or no chemical change and are taken up by the tissues and incorporated into them. In a previous chapter it has been noted that native protein may be absorbed as such at times and fail to reappear leading to the inference of utilization by the tissues. This, however, cannot be accepted as the usual procedure for all the food protein. Once in the blood stream as shown by parenteral introduction protein utilization apparently occurs through the intervention of enzymes which suddenly make their appearance in the circulation.

2. The proteins of the food are hydrolyzed and the products are absorbed and carried to the tissues.

3. The digestion products are synthesized into serum protein by the intestinal wall during the act of absorption, and the serum protein serves as pabulum for the tissues.

4. Deamination of the digestion products occurs previous to their entrance into the circulation. Since it is impossible to accept the hypothesis that unchanged protein is the form in which ingested protein is usually absorbed the next natural inference is that the proteoses and peptones are absorbed directly into the blood and conveyed to the tissues. In attempting to determine the correctness of this hypothesis the query has arisen

Are Proteoses and Peptones Present in the Blood?

In spite of the discovery of erepsin by Cohnheim and the consequent improbability of proteoses and peptone representing the usual form of final digestion products, some modern investigators have clung to the idea that it is in the form of proteoses and peptones that protein is absorbed. This view is based upon a number of investigations from which the assertion has been made that proteoses and peptones are present in the blood stream. The work of Neumeister led to the conclusion that proteoses and peptones are not found in the blood and it was not until 1903 through the

observations of Embden and Knoop that any doubt of Neumeister's view was entertained. In experiments designed to show the fate of proteoses and peptones when brought into contact with the intestinal wall Embden and Knoop were able to show the presence in the blood of substances having the properties of proteoses and peptones. They held that the mucous membrane of the intestine neither synthesized these substances to a larger molecule, as for example to a coagulable protein, nor were they hydrolyzed to the amino acid stage but, on the contrary, absorption took place directly. The results of Embden and Knoop were confirmed by some observers and discredited by others. Schumm for example was always unable to find a trace of proteose in the blood both under normal and abnormal conditions of health. Abderhalden and his coworkers maintained that the substances giving reactions for proteoses and peptones were present because of imperfect methods employed in the separation of the coagulable protein from the blood.

It has been pointed out by others, however, that there is a possibility of the presence in the blood of a protein naturally non-coagulable which would still give the reaction—the biuret test—significant under the experimental conditions for the presence of proteose or peptone. Zanetti described such a protein in the blood and found that it belonged to the group of mucoids. Among others, Howell believes in the existence in the blood of a protein possessing some of the characteristics of the proteoses and peptones, for example, non-

coagulability, which is not, however, identical with these substances. Bywaters has concluded that this body is a substance called by him sero-mucoid. In spite of these views Bergmann and Langstein and Kraus assert that small amounts of true proteose are present constantly. After feeding elastin Borchardt claimed to find elastin proteose in the blood stream. Upon repetition of this work of Borchardt, Abderhalden and Ruehl failed to give it confirmation. The explanation of Abderhalden and Oppenheimer that imperfect separation of coagulable protein is responsible for the proteose test was denied by Freund who maintained that the method employed by Abderhalden not only precipitated the coagulable protein but the proteose also.

From the foregoing brief review of only a few of the investigations carried through for the decision of the problem it is apparent that the whole question is in a chaotic state of contradictions and that a positive answer cannot be given. It may at least be said that positive proof of the presence of proteoses and peptones is still lacking. Perhaps one of the most convincing arguments against the existence in the blood of proteoses and peptones is derived from the work of Abderhalden and Pincussohn. They have demonstrated that just as with the parenteral introduction into the body of native protein so with proteose injection there is a development in the blood plasma of an enzyme capable of causing its disintegration to smaller molecules. Such an enzyme is not present in the

blood under ordinary conditions. If proteoses were present normally in the blood it is probable that this enzyme would also be a normal constituent of the blood.

The Synthesis, or Regeneration of Protein, by the Intestine

If it is accepted that protein is not absorbed in the form of proteoses or peptones the query naturally arises, In what *form* is it absorbed? An answer to this question must necessarily determine also the place of protein regeneration so long as the conception of nutrition obtains that metabolic changes in the organism demand the formation of new material to replace that broken down. If one maintains that protein gets into the blood stream in the form of a molecule larger than the amino acid, proteose or peptone molecule, it is self-evident that the intestine must be regarded as capable of synthesizing amino acids to protein. On the other hand if amino acids are regularly present in the systemic circulation, the *place* of protein regeneration must be relegated to the cellular elements of the different tissues.

Abderhalden. In the past various theories have been maintained. In view of the failure to find amino acids in the blood, Abderhalden put forward the view that the intestinal wall possessed the power during the act of absorption to synthesize the amino acids to proteins, probably serum proteins, to meet the needs of the

organism's requirements. The necessity for preliminary extensive disintegration of the food protein has been offered as follows: "Every species of animal—in fact every individual—has its specifically constituted tissues and cells. If the diet was always the same, the formation of the tissues might bear some close relation to the components of the food. The diet varies, however, and, especially in the case of human beings and the omnivora, is exceedingly diverse in nature and to make its organism independent of the outer world in the matter of food taken, it disintegrates the nutrient it receives, and utilizes those components which may be of service to it in building up new complexes."

Objections to this theory have been summarized by Cathcart as follows: "The view that the tissue proteins differ from one another, that they are specific bodies of definite constitution, and that, therefore, each requires a different amount and supply of building material is gradually being accepted. Abderhalden himself accepts this. What end then is served in having a single uniform pabulum formed when the demand is so varied? This is all the more questionable when it is remembered that there is no indubitable evidence which shows that one amino acid can be converted into another. Further, the belief is gradually gaining ground, as regards the protein requirements of the organism, that it is not so much the actual quantity as the quality of the protein supplied in the food, which is of importance. If the material supplied be uniform it necessitates a fresh breakdown by each tissue,

perhaps by each individual cell. Although the tissues all probably possess this power of breaking down protein material by means of their intracellular proteolytic enzymes, still the extra work involved seems to negative the immediate resynthesis hypothesis, especially when the hypothesis of the circulating digestion product postulates the presence of the individual food material in the blood. As already remarked, the mere failure to detect these products in the blood does not give adequate reason for concluding that they are not present. The tissues certainly do not break down in regular sequence, nor are they left to fall to pieces for lack of repair material. Repair is among the most active functions of all tissues. Must, then, a tissue of highly complex structure keep destroying and digesting plasma, picking out from the debris the nuclei which it requires and letting the rest go? (Why, and this destruction is admitted by Abderhalden, are the superfluous amino acids not found in the blood?) What happens, for instance, in the case of connective tissues with their demand for, say, glycine, where the food supply is not over-abundant as the circulation is poor, and the tissues not very suited for lymph perfusion? It will not do merely to say that there is no great breakdown of material here. Pflüger, in an interesting paper in which he combated this immediate resynthesis hypothesis, ascribed to the cells of the intestinal wall, with regard to the protein synthesis, the same capacity as the cells of all tissues, but denied that the synthesis of protein for the whole organism

was carried out there. He held that such a hypothesis was contrary to all existent knowledge of physiological assimilation."

One may query to what extent does immediate resynthesis take place? Are all the digestion products transformed into coagulable protein or are some selected and others rejected in part? The questions cannot be answered by the supporters of Abderhalden's theory. On the other hand, the intestine evidently is capable of exerting a marked selective action as to the type and amount of amino acid it shall absorb. The experiments of Abderhalden and his co-workers have indicated this. They fed gliadin to polyfistular animals and observed as the material traversed the gastro-enteric tract that tyrosine disappeared from the intestine whereas glutamic acid steadily increased in amount.

Freund. The idea of Freund is somewhat similar to the hypothesis advanced by Abderhalden except that he ascribes to the liver an important rôle in the subsequent breakdown of the protein. The protein digestion products are assumed by Freund to travel the portal circulation in the form of pseudo-globulin. The liver is unable to properly decompose protein unless it has first entered the blood stream by way of the intestine. This hypothesis carries with it the suggestion that the parenteral utilization of protein must be carried out through aid from the intestine, that the protein is excreted from the blood into the intestine, undergoes digestion, the products are absorbed, and

during the act of absorption are polymerized. This suggestion has been tested in different ways. One might expect that protein parenterally introduced into dogs, the intestine of which had been removed, would reappear in the urine. Körösy has failed to find more than traces of protein after parenteral injections of protein into animals without an alimentary tract. These observations tend to show that intestinal preparation of protein cannot be regarded as essential. The problem was attacked in another way by Abderhalden and London. They attempted to determine the excretion of protein into the intestine of polyfistular animals after parenteral introduction but failed to obtain any evidence of such a reaction. On the other hand, the excretion of substances into the intestine after parenteral injection is known. Thus, Abderhalden and Slavu have shown that iodine may find its way into the intestine when iodine-polypeptide combinations are injected subcutaneously.

Hofmeister. It was the view of Hofmeister that the leucocyte is intimately associated with protein regeneration. The idea undoubtedly originated from the marked leucocytosis which occurs after meals and Hofmeister thought that peptone after absorption was changed in some unknown manner into protein by the leucocytes or else through the agency of adenoid tissue. Later, the lymphocyte was selected as the specific form of leucocyte responsible for protein synthesis. This theory, however, finds few supporters today. Perhaps the best criticism of the leucocyte synthesis theory has

been offered by Halliburton. "He pointed out that the number of the lymphocytes was not commensurate with the work to be done. He calculated that a man of eighty kilos had about four kilos of blood of which some 40 per cent was in the form of corpuscles, that is about 1600 grams. Now as the ratio of white corpuscles is about 1 : 500 it means that about 3.2 grams of leucocytes are present. Of this amount lymphocytes form at most 30 per cent, and therefore in the blood there would be about one gram of lymphocytes. If this amount were doubled during digestion 'it is difficult to see how two grams of lymphocytes can tackle the enormous burden which every meal must impose upon them.'" (Cathcart.)

What Is the Evidence for the Synthesis of Protein?

The Synthetic Action of the Gastric and Intestinal Mucous Membranes.

Hofmeister has ascribed to the stomach mucous membrane the property of synthesizing protein from proteoses. An outline of his experiment follows—at the height of digestion a dog was killed and its stomach and contents divided equally into two parts. One part was immediately placed in boiling water to stop all enzyme and cellular activity and the other portion was placed in an incubator for a period of two hours. The amount of proteose and peptone present in each part was then determined. In the por-

tion placed in the incubator, there was an almost complete disappearance of proteose and peptone, which of course could not be ascribed to further decomposition since gastric juice does not hydrolyze proteoses and peptones to amino acids, at least during such a short period. The conclusion drawn was that the proteoses and peptones disappeared because of their synthesis to protein. In other experiments Hofmeister demonstrated that the intestine possesses the same property. Glaessner repeated and confirmed Hofmeister's investigation. On the other hand, Embden and Knoop failed to find any evidence of protein synthesis. They employed the normal intestine and also the intestine from which pancreatic juice was excluded by ligature of the duct. The evidence for resynthesis of protein in the gastric or intestinal mucous membranes is not convincing and one must obtain other than negative evidence before the idea of such a protein resynthesis can be accepted.

Plastein Formation. It has been observed repeatedly that when solutions of proteoses are brought into contact with rennin a precipitate called plastein forms. Various views as to its formation have been held. It has been assumed by some that plastein is a new synthetic product formed from the proteoses—a new protein, by others a resynthesis of the proteoses to the original protein from which they were derived, and by still others as a digestion product on its way to complete solution. The results of the most searching investigations concerning the nature of plastein incline

one to the belief that this substance is of the nature of proteose rather than that of a complete protein so that plastein formation affords little or no evidence for the support of the existence of protein synthesis.

The Synthetic Action of Pepsin and Trypsin

It has been demonstrated by A. E. Taylor that by the long continued action of trypsin of the clam liver upon concentrated protamine digestion products a reformation of protamine takes place. The quantity reformed is very small in comparison with the original amount of protamine digestion products. In a similar manner Robertson has found the synthesis of a paranuclein by the action of pepsin upon concentrated casein digestion products. These results lead to the suggestion that trypsin and pepsin may possess a twofold action, a disintegrative influence and a synthetic action in accord with the idea of the reversibility of enzymes. If the synthetic action in the intestine is as slow as that shown in the experiments just cited little value can be assigned to them as aids in the regeneration of protein in the body for the influence could be observed only after the influence had continued for several months.

The evidence for the synthesis of protein in the intestinal wall is all of an indirect nature. If the adherents of that theory could demonstrate an increase of protein in the blood after an ingestion of protein

their argument might be established. This they have failed to do.

Deamination

The failure to demonstrate the presence of amino acids in the blood of the higher animals during digestion led to the conception that the amino acids are deaminated, that is, ammonia is split off while passing the intestinal wall, this deamination being regarded as the first stage in the catabolism of the amino acids. This possibility was suggested by the work of Cohnheim upon certain of the lower forms of animal life in which he showed the giving off of ammonia by the intestine after addition of amino acids, and derived support from the older work of Nencki and others who showed that the ammonia content of the portal blood was greater than that of the arterial during digestion. It has been assumed that as result of this process of deamination the ammonia split off is transformed by the liver into urea and so quickly eliminated by the kidneys. Such a view has been adopted as explanatory for the long known rapid rise in urea excretion following protein ingestion.

It has been shown by Lang that deamination is a property of a great many tissues of the body but it is probable that certain of them possess a selective action in this respect for some tissues deaminate certain of the amino acids much more readily than others. In particular the intestine and liver seem to possess this action in a high degree. To the liver a great import-

ance has been attached as a deaminating agent and during recent years discussion of the so-called defective or insufficient deamination in a series of pathological conditions has come into vogue.

In accordance with this idea amino acids have been administered as a test for the functional activity of the liver. Glaessner has shown that normal liver tissue is capable of transforming definite amounts of specific amino acids into urea. In a series of experiments he has shown that in various diseased conditions of the liver, such as fatty liver, in syphilis, cirrhotic liver, and a phosphorus poisoned liver a failure to convert amino acids into urea and a consequent output of amino acids in the urine took place.

That deamination is undoubtedly an important intracellular activity may be derived from a series of experiments in which amino acids have been fed and their fate determined. Thus with arginine most of the nitrogen reappears as urea. Probably through the intervention of the enzyme, arginase, a splitting of arginine into urea and orinthine occurs and by deamination of the latter more urea is formed. Again, after administration of alanine, lactic acid in the urine has been observed. With the purines also it may be shown that a splitting off of ammonia occurs. One may accept without hesitation that the function of deamination is an important activity of cell life. The contention that certain organs or tissues possess this function more specifically than others has been a matter of controversy. The rôle played by the intestine in

this regard is especially to be considered, correlated as it has been with the explanation of the form in which protein digestion products are absorbed.

The recent observations of Folin and Denis and others have rendered untenable the hypothesis that deamination by the intestine is the first stage in the catabolism of amino acids. They demonstrated that during the absorption of amino acids from the intestine there was no increase in ammonia or urea of the blood and they further showed that the ammonia of the portal blood is produced in large measure by the products of putrefaction in the large intestine. The retirement of the theory of intestinal deaminization to account for the apparent absence of amino acids in the blood carries with it also the untenability of the idea that the liver is specifically concerned in the formation of urea. To quote the authors: "In the absence of satisfactory proof that deaminization and urea formation is localized we are not justified in assuming that the process is a specialized process in the sense of being confined to some particular organ. In other words, so far as we yet know, the urea forming process is a characteristic of all the tissues and by far the greatest amount of urea is therefore probably formed in the muscles. The negative results, so far as any localized urea formation is concerned, is almost satisfactory proof that there is none, for if there were one central focus from which all or nearly all of the urea originated we could scarcely have failed to find it."

Are Amino Acids Found in the Blood?

Opposed to the investigators advancing the regeneration of protein immediately after absorption is a second group of men who have long believed that amino acids are absorbed into the blood. The great difficulty has been to demonstrate their presence. A large number of experiments have been devised in various ingenious ways to overcome the difficulties attendant upon such a procedure. Many investigators have obtained partial evidence of the presence in the blood stream but an actual isolation and identification of individual amino acids was for a long time lacking.

The failure to obtain definite proof of the amino acids in the blood has been due in large measure to the inadequacy of the methods available. At any one moment the quantity of these substances in a determined sample of blood must be exceedingly small.

Moreover, one must remember that the formation of amino acids in the intestinal tract is a gradual process and not of the nature of an explosion so that the quantity of amino acids available for passage into the blood during a given period must be relatively small. The rapidity of circulation is another factor to be taken into consideration. It has been shown that in the portal vein of the dog the blood travels at the rate of about 150 cc. per minute. Pflüger has estimated that for human beings a maximum rate of absorption of protein may be represented at 1.14 gram protein per kilo per hour. If the 1.14 gram protein absorbed

per hour is compared to the volume of blood in which it would necessarily be dissolved we find that the protein would be present in the concentration of 0.12 per cent. Another difficulty arises from the fact that the blood is a fluid already containing about 3 per cent of coagulable protein and also nitrogen to a smaller extent in other forms.

Certain fairly definite indications, however, have led many physiologists to maintain a belief that protein is absorbed and is taken up by the cells in the form of amino acids.

Determination of the "residual nitrogen" of the blood, that is, the difference between the total nitrogen and that representing coagulable protein has shown that after meals there is a slight gain both in the portal blood and that of the systematic circulation. This increase of nitrogen may be attributed to the absorbed amino acids or polypeptides, but in view of the possible existence of non-coagulable proteins in the blood it cannot be accepted as proof positive. A second method for the same endeavor has been the formation of amino acid compounds by use of β-naphthalene sulphochloride. Shaken with the fluid obtained after separation of the serum proteins either by coagulation or by means of dialysis precipitates have been obtained with this reagent, strongly indicating the presence of amino acids but the failure of the precipitate to assume a crystalline form has made impossible a positive identification of amino acids. Cohnheim by observations with the isolated intestine of the octopus was able

to prove absorption and the existence in the blood of certain amino acids but failed to detect these substances when experiments were carried out on the intact animal.

By the elaboration of new methods, Folin and Denis and Van Slyke and Meyer have been able to prove the entrance of amino acids into the blood stream. Later, Abderhalden, the chief opponent of the idea of amino acid absorption, was successful in isolating from the blood several of the individual amino acids by the employment of great volumes of blood. The absorption of protein in the form of amino acids having thus been established the question next arises what becomes of them? It was soon proved that there was a rapid disappearance of amino acids from the circulation and this fact made pertinent the queries: "Are they decomposed in the blood: are they chemically incorporated into the complex molecules of the tissue proteins; or are they merely absorbed by the tissues in general, or by certain tissues in particular, without undergoing any immediate change?" These questions have been fully answered by Van Slyke and Meyer in experiments designed to follow the fate of the amino acids after absorption. It was found that the amino acids are absorbed by the tissues without undergoing any immediate chemical change. This absorption though rapid is never complete, the blood always containing a small quantity of amino acids. It would appear from this that there is an equilibrium between the amino acids of the blood and of the tissues. The way

in which amino acids are taken up by the tissues and held by them is still undetermined.

In a later communication the same investigators have attempted to determine the fate of amino acids after absorption by the tissues and selected the changes occurring in the liver. Amino acids absorbed by the liver rapidly disappear. In explanation of this observation several possibilities exist: 1. The amino acids may be excreted through the bile. This view, however, is not probable since the quantities of amino acids in the bile and urine were entirely too small to account for the amount that disappeared from the liver. 2. A second possibility is that the amino acids are transferred to other tissues. This hypothesis is also highly improbable since none of the other large organs show a greater avidity for amino acids, yet three or four hours after injection of amino acids other organs usually contain more amino acids than the liver. 3. The absorbed amino acids are synthesized into body protein in the liver. The possibility cannot be definitely decided at present. 4. The amino acids are deaminated with formation of urea or ammonia. In all probability a portion of the amino acids which disappears from the liver reappears in the urine as urea.

The disappearance of amino acids from the liver is more rapid and complete than is true for other tissues like the kidney, intestine, pancreas, and spleen. From the muscles the amino acids disappear very slowly. As a summary of the whole question one may quote the

words of Van Slyke and Meyer: "The amino acids, with perhaps some peptides, from the intestine enter the circulation, from which they are immediately absorbed by the tissues. The power to take them up from the blood stream is common to all the tissues, but is limited. The muscles of the dog, for example, reach the saturation point when they contain about 75 mgm. of amino acid nitrogen per 100 grams. The liver, however, continually desaturates itself by metabolizing the amino acids that it has absorbed, and consequently maintains indefinitely its power to continue removing them from the circulation so long as they do not enter it faster than the liver can metabolize them. When the entrance is unnaturally rapid, as in our injection experiments, or when the liver is sufficiently degenerated, as observed clinically in some pathological conditions, the kidneys assist in removing the amino acids by excreting them unchanged. Death may result when the above agencies for preventing undue accumulation of protein digestion products are over-taxed.

"In regard to the synthesis of tissue proteins it appears reasonable to believe that, since each tissue has its own store of amino acids, which it can replenish from the blood, it uses these to synthesize its own proteins."

Concerning the manner in which the free amino acids are utilized by the tissues two possibilities may be assumed, and according to Van Slyke and Meyer these are: 1. The amino acids serve as a reserve energy supply, like glycogen, or as a reserve of tissue-building

material. In either case the supply would be depleted if not renewed from the food. 2. The amino acids are merely intermediate steps in both the construction and breakdown of the tissue proteins. In this case they could originate, not only from absorbed food products, but also from autolyzed tissue protein: starvation would not result in a disappearance of the amino acid supply of the tissues, and might even increase it. To determine the correctness of one or the other of these hypotheses the authors mentioned above analyzed the tissues of animals in various states of nutrition. The results are in harmony with the second hypothesis, for free amino acids of the tissues tend to increase in starvation rather than to disappear. The investigators have summarized their views regarding this in the following words: "The amino acids appear, therefore, to be intermediate steps, not only in the synthesis, but in the breaking down of body proteins. Otherwise, in order to explain their maintenance in the tissues during starvation, one would be forced, contrary to the conclusions of all experimental work on the subject, to assume that they are inert substances lying unchanged for long periods, even when most urgently needed to build tissue or supply energy. The maintenance of the amino acid supply by synthesis, from ammonia and the products of fats or carbohydrates, seems excluded. The supply of raw material in the form of fat and carbohydrates nearly disappears during starvation, and the ammonia could originate only from broken-down protein, as the normal store of ammonia nitrogen is

only a fraction of that of the free amino acids. These considerations, and the self-evident wasting of starved tissues, point strongly to autolysis as the main source of the free amino acids in the fasting body."

"The failure to increase the free amino acid content of the tissues by high protein feeding indicates, furthermore, that when nitrogen is retained in the organism it is not to an appreciable extent, as stored digestion products, but rather as body protein."

These results, and the consequent inference from them, have made void all the older theories of metabolism and it is becoming more and more evident that in any consideration of protein transformations within the organism in health or in disease amino acids are the substances which demand attention. This is the age of amino acid metabolism and at present the investigations are being narrowed down to the point of the determination of what actually occurs with the individual amino acids and what special rôle in nutrition each may play.

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CHAPTER V

THEORIES OF PROTEIN METABOLISM

Under the term protein metabolism are included all the processes in the animal organism concerned with the fate of protein whether introduced as food or serving as tissue substance. The metabolic changes are divisible into two distinct phases known as *anabolism,* or building up processes, and *catabolism,* or destructive processes. Although it is definitely recognized that metabolic activity is manifested in two diametrically opposed directions, the successive stages in either process are but vaguely understood. Only the starting point and the final end products in each instance can be stated with certainty, although here and there individual stages in the processes under discussion point in one or another direction, and thus give indication of the type of activity that must have gone before. For the unravelling of the mystery the first requisite is a clear conception of the problem. In the words of Liebig: "If we take the letters of a sentence which we wish to decipher, and place them in a line, we advance not a step towards the discovery of their meaning. To resolve an enigma, we must have a perfectly clear conception of the problem. There are many ways to the highest pinnacle of a mountain; but

those only can hope to reach it who keep the summit constantly in view. All our labor and all our efforts, if we strive to attain it through a morass, only serve to cover us more completely with mud; our progress is impeded by difficulties of our own creation, and at last even the greatest strength must give way when so absurdly wasted."

The development of knowledge in science succeeds best when an hypothesis is formulated as a basis for investigation. By holding fast to that which is proven as fact and discarding that which is shown to be contrary to fact is real progress made. This, indeed, has been the case in the history of protein metabolism, as may be seen in the following pages where is traced the evolution of ideas concerning it.

Liebig

The first clearly defined theory of protein metabolism was that enunciated by Liebig who assumed that protein material undergoes little or no chemical change previous to its introduction into the blood stream and its assimilation by the tissues. "According to this theory, the plant holds a position intermediate between the mineral and animal world. The animal is incapable of assimilating the compounds stored up in inorganic nature. To render these compounds subservient to the purposes of animal life they may have to undergo a preliminary preparation within the living organism of the plant. The simple mineral molecules are thus con-

verted into molecules of a higher order, fit to serve in building up and maintaining alive the body of the animal." "How admirably simple after we have acquired a knowledge of this relation between plants and animals, appears to us the process of formation of the animal body, the origin of its blood and organs! The vegetable substances, which serve for the production of blood, contain already the chief constituent of blood ready formed, with all its elements." "The true starting point for all the tissues is, consequently, albumen; all nitrogenized articles of food, whether derived from animal or from the vegetable kingdom, are converted into albumen before they can take part in the process of nutrition."

According to Liebig digestion is merely a process whereby food becomes changed to a soluble condition capable of absorption without transformation of its identity. This soluble albumen is built up into organized tissue previous to its degradation (an idea later adopted by Pflüger). Thus we read: "There can be no greater contradiction, with regard to the nutritive process, than to suppose that the nitrogen of the food can pass into the urine as urea, without having previously become part of an organized tissue; for albumen, the only constituent of blood which, from its amount, ought to be taken into consideration, suffers not the slightest change in passing through the liver or kidneys; we find it in every part of the body with the same appearance and the same properties."

Liebig divided all foods into two groups, the nitro-

genous, or *plastic,* foods, and the non-nitrogenous or *respiratory* foods. In accordance with this classification plastic foods were tissue formers and supplied energy for muscular activity; the respiratory foods, on the other hand, were essential for the respiratory act and the constant temperature of the body, but could not be transformed into organized tissue.

Voit

The fundamental conception of Voit (1867) was that all protein in the body is not decomposed with equal ease. In accordance with this idea he divided the protein material of the body into two groups, the *organized* or *tissue* protein, that built up into living protoplasm and difficult of disintegration and, secondly, *circulating protein* existing in the fluids and tissues of the organism without being an integral part. The circulating protein may be more easily and readily destroyed than the organized or tissue protein. In his classic experiment designed to show the difference of metabolism between tissue protein and circulating protein, Voit allowed a well-fed dog to starve for several days. He demonstrated that under these circumstances there is at first an abundant decomposition of protein material which is later followed by a period during which very little protein is catabolized. His interpretation of these facts was to the effect that during the time when a large protein disintegration obtained only circulating protein was destroyed, whereas

in the later stages the greatly diminished uniform protein metabolism was that of the organized or tissue proteins.

In his theory Voit assigned to the *cells* the function of utilizing proteins, the older view that metabolism took place in the blood having been discarded. From the fluids bathing the tissues food or circulating protein is drawn within the cells and there transformed. On the other hand, a certain small amount of tissue protein is constantly dying and is replaced by circulating protein, thus becoming eventually living protoplasm. "The tissue-elements constitute an apparatus of a relatively stable nature, which has the power of taking proteins from the fluids washing the tissues and appropriating them, while their own proteins, the tissue proteins, are ordinarily catabolized to only a small extent, about 1 per cent daily." (Voit.) "By an increased supply of proteins the activity of the cells and their ability to decompose nutritive proteins are also increased to a certain degree. When nitrogenous equilibrium is obtained after an increased supply of proteins, it indicates that the decomposing power of the cells for proteins has increased so that the same quantity of proteins is metabolized as is supplied to the body. If the protein metabolism is decreased by the simultaneous administration of other non-nitrogenous foods, a part of the circulating proteins may have time to become fixed and organized by the tissues, and in this way the flesh of the body increases. During starvation or with a lack of protein in the food the

reverse takes place, for a part of the tissue proteins is converted into circulating proteins, which are metabolized, and in this case the flesh of the body decreases." (Hammarsten.)

Pflüger

In 1893 Pflüger severely criticised the theory of Voit and offered another in its place. In its essence the theory of Pflüger rests upon the hypothesis that food protein must become living protoplasm before it can be utilized for the needs of the body. In accordance with this idea he assumed that food protein is catabolized with great difficulty whereas living protoplasm is in a state of continual unstable equilibrium leading to any easy oxidation or decomposition of its protein. Pflüger's theory rests upon experiments carried through by his pupil Schöndorff. It was shown by Schöndorff that when the blood from a starving dog was passed through the hind limbs and liver of a well-fed animal the urea of this blood was increased. On the other hand, no increase of urea could be obtained when blood, whether of starved or well-fed dogs, was passed through the hind limbs and liver of a starved dog. From the results Pflüger argued that the determining factor in protein catabolism is the state of nutrition in the tissue cells and not the circulating protein.

Although in general Pflüger appeared to disprove many of the points in Voit's theory, one positive evidence stands out clearly in favor of Voit's theory, and

that is the fact of the rapidity with which large quantities of protein are catabolized in the body. In a few hours great quantities of protein may be disintegrated as judged by the corresponding increase in urinary nitrogen. It is hardly probable that living protoplasm could be synthesized so rapidly and so much of it be so quickly destroyed again. This is the more incredible since the same fact applies irrespective of the previous state of nutrition of the organism.

In 1905 Folin subjected the experiments of Schöndorff to a searching criticism and pointed out that the evidence furnished by them was by no means unassailable. Upon studying the details of one of Schöndorff's experiments, Folin found that the actual increase in urea nitrogen in the transfused blood amounted to less than one-tenth of 1 per cent instead of 125 per cent as calculated by Schöndorff. "Considering the numerous sources of error and uncertainty necessarily attached to an experiment of this kind, it seems very strange that the extraction of 25 mgm. of urea-nitrogen from the hind limbs of a dog killed while engaged in digesting 700 gm. of meat should be accepted as proving not only that protein catabolism did occur during the experiment, but also that it occurred in the bioplasm and not in the circulating protein."

No direct evidence has been obtained to prove or disprove the one or the other of these last two widely divergent theories. The distinction between tissue protein and food protein is probably one of degree rather than of kind.

Kassowitz

Kassowitz in 1904 put forth the view that it was scarcely probable that a substance would serve both as reconstructive material for disintegrated cells and as a source of energy. According to his ideas food protein is not merely transformed into living protoplasm by some obscure rearrangement but there is an actual synthesis with fat and carbohydrate to form living bioplasm. Like Pflüger he adopts the view that only "organized" protein is oxidized.

In metabolism there are two types of protoplasmic disintegration: the inactive, whereby the protoplasm formed from food protein during rest is immediately changed or broken down into non-nitrogenous storage materials (glycogen and fat) and urea; the active, by which under the influence of stimuli which induce muscular contractions, the protein nucleus of the disintegrating protoplasm molecule is left intact so that it may serve for the resynthesis of protoplasm with fresh non-nitrogenous compounds. (Mendel.)

Speck

In the theory of Speck (1903) the view is held that two forms of protein exist but that the catabolism of organized protein is quite different from that of the unorganized protein. That portion of food protein (unorganized) not employed for the building up of living tissue, is split into two portions, first, a nitrogenous part, which is rapidly converted into urea,

and a nitrogen free residue, serving as a ready source of energy.

On the other hand, after death of cells, the tissue or organized protein, although also broken down into two parts, finds a destiny unlike the products of food protein disintegration. Tissue protein splits into a nitrogen-containing and a nitrogen-free portion. Under normal conditions the nitrogen-free part is transformed to glycogen or fat which may be utilized for purposes of energy. The portion containing nitrogen is not broken down at once into urea but it leads to the formation of a variety of substances which play important rôles in metabolism but are finally excreted as urea. In the decomposition of tissue protein Speck assigned to oxygen deficiency an exceedingly important part.

Rubner

In Rubner's theory of protein metabolism it is maintained that a study of metabolism cannot be considered separately from the study of heat production. According to Rubner, therefore, metabolism must be studied in connection with the exchange of energy. In all of the metabolic changes undergone by protein in the body reference is made to the accompanying production of heat. Rubner believes in a "store" protein which may be compared to Voit's "circulating" protein, and in a "wear and tear quota" necessary for the repair of tissue waste. He assumes that most of the protein after absorption is rapidly split into two

parts, one nitrogen-free, the other containing nitrogen. Inasmuch as the nitrogen-containing part plays little rôle in energy exchange, its fate is left somewhat indefinite. The part free from nitrogen forms the dynamic quota of the protein ingested. When protein is disintegrated into its two parts mentioned above, there occurs a certain liberation of heat which is of no value to the body cells and is therefore lost. This liberation of energy has been called by Rubner the "specific dynamic action" of protein.

"A highly speculative hypothesis explained how the various changes took place. All protoplasm was not regarded as being of the same type, one kind might be thermolabile, another thermostable, but all varieties had in common a certain molecular grouping which acted as a kind of nucleus to which other protein groups (for example those which were thermostable or thermolabile) could attach themselves. The mechanism of the energy exchange, which is characteristic of activity, was effected by a distinct vibratory movement of the whole or a definite part of the protoplasm. Owing to the specific oscillation, the protoplasm had the power of bringing about the breakdown of contiguous foodstuffs. The 'affinities' (specific oscillations) must be of a specific nature for each tissue and were probably somewhat akin to ferment action. Thus, in diabetes, the 'affinities' which brought about the breakdown of carbohydrates, were for some reason or other in a state of suspension, inoperative or actually destroyed, whereas those which

dealt with the catabolism of fat were active. The direct effect of the approximation of the foodstuffs to the 'affinities' resulted in an atomic rearrangement and the entry of oxygen. The potential energy of the foodstuff now became available and caused a complete alteration in the 'affinities'; an absorption of energy into the living substance took place at the moment of the catabolism of the foodstuff. The internal oscillations and changes in the cells, however, gradually used up all the energy, which was converted into heat and lost, and there was a return to the original condition, the 'affinities' being again ready to begin work. The rate of the change depended on the nature of the living substance, the temperature, nervous influences, and the conditions of the organism itself." (Cathcart.)

Folin

It was Folin's conception that "the laws governing the composition of the urine represent only the effects of other more important laws governing the catabolism of protein in the animal organism" which led him to determine these laws under widely differing conditions of diet. His interpretation of protein metabolism on the basis of observed variations in the percentage composition of the urine has stood as the almost universally accepted theory of protein metabolism of the present period.

Previous to his investigation only lengthy and none too accurate methods were in use for the estimation

of the urinary constituents. He, therefore, first devised a method, in each instance short and accurate, for the estimation of every important nitrogenous constituent of the urine, together with methods for the determination of sulphur containing compounds, and, secondly, with the aid of these methods made complete analysis of normal urines. In order to make the factor of food protein as evident as possible, diets rich in protein were fed and were succeeded by rations markedly deficient in nitrogenous substances although containing a sufficiency of energy yielding substances.

As showing the wide range of variation on the two diets a typical example of the urinary composition follows:

	Nitrogen rich diet	*Nitrogen poor diet*
Volume of urine	1170 c.c.	385 c.c.
Total Nitrogen	16.8 grams	3.60 grams
Urea-Nitrogen	14.7 grams = 87.5%	2.20 grams = 61.7%
Ammonia-Nitrogen	0.49 gram = 3.0%	0.42 gram = 11.3%
Uric acid-Nitrogen	0.18 gram = 1.1%	0.09 gram = 2.5%
Kreatinine-Nitrogen	0.58 gram = 3.6%	0.60 gram = 17.2%
Undetermined Nitrogen	0.85 gram = 4.9%	0.27 gram = 7.3%
Total SO^3	3.64 grams	0.76 gram
Inorganic SO^3	3.27 grams = 90.0%	0.46 gram = 60.5%
Ethereal SO^3	0.19 gram = 5.2%	0.10 gram = 13.2%
Neutral SO^3	0.18 gram = 4.8%	0.20 gram = 26.3%

The general laws deduced by Folin as a result of urinary analysis are:

1. Kreatinine. The absolute quantity of kreatinine eliminated on a meat-free diet is a constant quantity,

different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated.

2. Uric Acid. When the total amount of protein metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the per cent of the uric acid nitrogen in terms of the total is, therefore, much increased.

3. Ammonia. With pronounced diminution in the protein metabolism (as shown by the total nitrogen in the urine), there is usually, but not always, and therefore not necessarily, a decrease in the absolute quantity of ammonia eliminated. A pronounced reduction of the total nitrogen is, however, always accompanied by a relative increase in the ammonia-nitrogen, provided that the food is not such as to yield an alkaline ash.

4. Urea. With every decided diminution in the quantity of total nitrogen eliminated, there is a pronounced reduction in the per cent of that nitrogen represented by urea. When the daily total nitrogen elimination has been reduced to 3 gm. or 4 gm. about 60 per cent of it only is in the form of urea.

5. Inorganic Sulphates. Decided diminutions in the daily elimination of total sulphur are accompanied by reductions in the per cent of the sulphur present as inorganic sulphates. The reductions are as great as in the case of urea.

6. Neutral Sulphur. The neutral sulphur elimina-

tion is analogous to that of the kreatinine. It represents products which in the main are independent of the total amount of sulphur eliminated or of protein catabolized.

7. Ethereal Sulphates. The ethereal sulphates represent a form of sulphur metabolism which becomes more prominent when the food contains little or no protein.

Folin concludes that neither the theory of Voit nor that of Pflüger can be correct for these theories do not harmonize with the above laws governing the composition of the urine. With respect to his own views he says: "We have seen (from the tables) that the composition of urine, representing 15 gm. of nitrogen, or about 95 gm. of protein, differs very widely from the composition of urine representing only 3 gm. or 4 gm. of nitrogen, and that there is a gradual and regular transition from the one to the other. To explain such changes in the composition of the urine on the basis of protein catabolism, we are forced, it seems to me, to assume that catabolism is not all of one kind. There must be at least two kinds. Moreover, from the nature of the changes in the distribution of the urinary constituents, it can be affirmed, I think, that the two forms of protein catabolism are essentially independent and quite different. One kind is extremely variable in quantity, the other tends to remain constant. The one kind yields chiefly urea and inorganic sulphates, no kreatinin, and probably no neutral sulphur. The other, the constant catabolism, is largely repre-

sented by kreatinin and neutral sulphur, and to a less extent by uric acid and ethereal sulphates. The more the total catabolism is reduced, the less prominent become the two chief representatives of the variable catabolism."

"The fact that the urea and inorganic sulphates represent chiefly the variable catabolism does of course not preclude the possibility that they also represent to some extent the constant catabolism."

In accordance with these two types of catabolism Folin has furnished suitable names. The protein metabolism which tends to be constant is *tissue* metabolism, or *endogenous* metabolism; the other, the variable protein metabolism, is the *exogenous* or intermediate metabolism.

Instead of assuming, as did Voit and Pflüger, that the same type of decomposition, i.e., oxidation, occurs with protein as with fats and carbohydrates, Folin advances the view that the disintegration of protein in catabolism is produced in large measure by a series of hydrolytic splittings, nitrogen being split off as ammonia.

It is further shown that contrary to the ideas of Voit and Pflüger, extensive formation of urea does not occur in the muscles. Folin believed (1905) that the nitrogenous cleavage products formed in the alimentary canal from food protein are denitrogenized, probably in the intestine, the ammonia split off, carried to the liver, built up into urea and eliminated. The non-nitrogenous residue is in part converted into

carbohydrates. "The chief reason why the nitrogenous splitting products produced by the digestive enzymes are universally assumed to be reconverted into albumin is the teleological one. The food proteins are tissue builders and the organism must not waste them. The fact that the muscle tissues of normal men do not increase when the protein of food is increased, but that all of the nitrogen of such protein is at once eliminated, has not been sufficiently considered in this connection. The only adequate teleological explanation of this fact is that this nitrogen is not needed for the building of new tissues. It is not needed because the organism cannot enlarge indefinitely, and because after it has attained its full growth the daily waste of tissue is small. Yet when more nitrogen than the organism needs is furnished with the food, we find that the protein containing it is still absorbed up to the limit of the digestive capacity." "The greater part of the protein furnished with standard diets like Voit's, i.e., that part representing the exogenous metabolism, is not needed, or to be more specific, its nitrogen is not needed. The organism has developed special facilities for getting rid of such excess of nitrogen so as to get the use of the carbonaceous part of the protein containing it. The first step in this process is the decomposition of protein in the digestive tract into proteoses, amido acids, ammonia, and possibly urea. The hydrolytic decompositions are carried further in the mucous membrane of the intestines, and are completed in the

liver, each splitting being such as to further the formation of urea."

"In these special hydrolytic decompositions, the result of which is to remove the unnecessary nitrogen, we have an explanation of why and how the animal organism tends to maintain nitrogen equilibrium even when excessive amounts of protein are furnished with the food. This excess of protein is not stored up in the organism, as such, because the actual need of nitrogen is so small that an excess is always furnished with the food. . . ."

Present-Day Theory of Metabolism

The proof of the presence of amino acids in the blood through the investigations of Folin, Van Slyke, and others has rendered necessary some slight modification of our views concerning metabolic processes. Although Folin in his original theory foretold the probable importance of the lower protein decomposition products it was not until the actual presence of these substances in the blood and tissues was demonstrated that acceptance of this idea was general. Since it has been proven beyond question that amino acids are normally absorbed directly into the blood from the intestine and are distributed to the tissues, it is assumed that each tissue rebuilds itself from the mixture of amino acids thus received. That portion of amino acids which is not necessary for synthesis is changed into urea and carbonaceous residues presumably by a

process of deamination. The carbon remainders may be transformed into carbohydrate or in other ways changed so as to yield energy and heat. Protein material broken down within the tissues undoubtedly suffers a series of hydrolytic cleavages, resulting in the formation of amino acids and the latter presumably undergo the same fate as those produced from food protein.

According to this view protein synthesis is not restricted to any one organ or tissue but all possess the same property. Urea formation also can no longer be assigned to the liver or some special urea-forming organ, but on the other hand every tissue probably is capable of forming this substance.

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CHAPTER VI

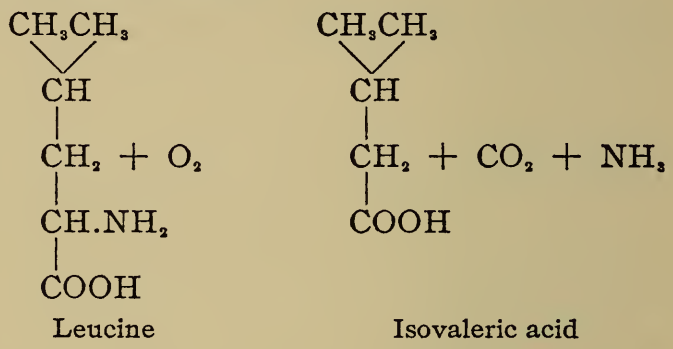
THE FURTHER FATE OF AMINO ACIDS

It is well recognized that a large part of the amino acids of the food is eliminated from the body in the form of urea, carbon dioxide, and water. The various amino acids presumably undergo a variety of chemical changes previous to their excretion as the simple products mentioned above. Also the ease with which these transformations take place is different for the individual amino acids. The steps leading to the ultimate fate of some are quite clear, of others it is very obscure or entirely unknown. The unlike ease of transformation of amino acids into urea has been shown by intravenous injection. Thus glycocoll and leucine yield urea more or less completely whereas alanine, cystine, aspartic and glutamic acids are not readily catabolized.

In general the first step in the metabolism of amino acids is that of oxidative deamination—a splitting off of ammonia with an accompanying oxidation. For any straight chain amino acid the reaction occurring may be represented as follows:

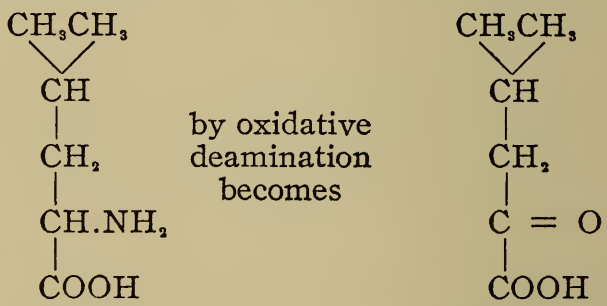
$$R.CH_2.CH.NH_2.COOH + O = R.CH_2.COOH + CO_2 + NH_3$$

The CO_2 and urea are then synthesized to form urea. This synthesis may occur according to our present views in any active tissue or organ. Taking leucine as a specific example of oxidative deamination we have the reaction following:



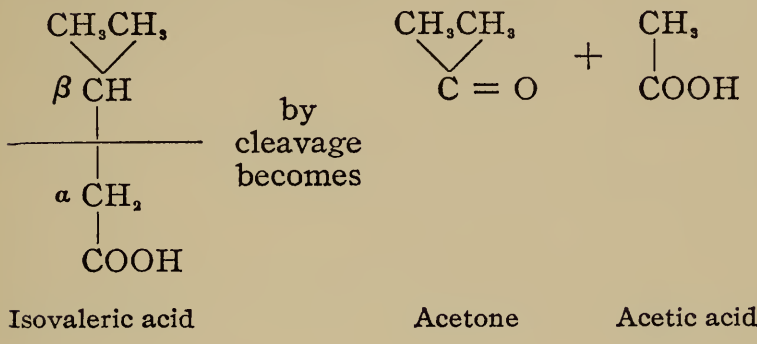
Leucine — Isovaleric acid

It has been shown that under suitable conditions leucine, for example, may yield acetone. In order to explain the chemistry of this change it becomes necessary to introduce the intervention of a type of acid known as a ketone acid, that is, one possessing the ketone group, C = O. Leucine by oxidative deamination may be changed to a ketone acid.



Leucine — Ketone acid

The ketone acid is then transformed to a lower fatty acid, isovaleric acid, by cleavage of CO_2.



Isovaleric acid Acetone Acetic acid

By cleavage of isovaleric acid between the $\alpha + \beta$ carbon atoms acetone and acetic acid may be formed, both of which may finally yield $CO_2 + H_2O$.

Another possibility of the transformation of straight chain amino acids is first the formation of hydroxy-amino acids, then oxidative deamination with the subsequent splitting off of CO_2 from the nitrogen free residue, or fatty acid, and the final direct change of the latter to $CO_2 + H_2O$, thus:

$R.CH_2.CH.NH_2.COOH$ is first changed to

$R.CH_2.C(OH).NH_2.COOH$

Hydroxy-amino acid

then oxidative deamination follows with the formation of a ketone acid.

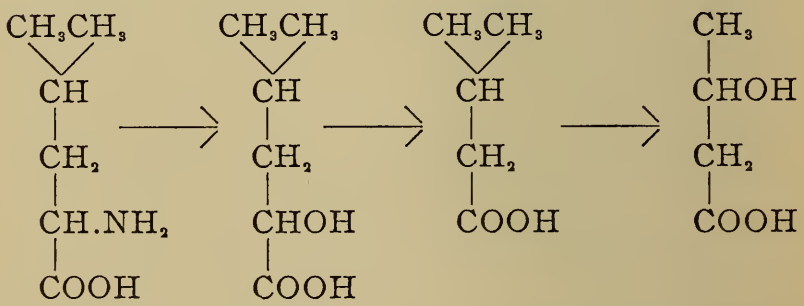
$R.CH_2.C(OH).NH_2.COOH$
Hydroxy-amino acid
$R.CH_2.CO.COOH + CO_2 + NH_3$
Ketone acid

By cleavage of CO_2 this becomes a fatty acid with less carbon atoms.

$R.CH_2.CO.COOH - CO_2 = R.CH_2.COOH$
Ketone acid

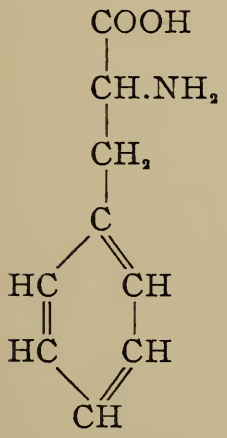
By further cleavage this fatty acid is changed to $CO_2 + H_2O$.

In an abnormal organism, such as that of the diabetic, leucine may yield beta-oxybutyric acid instead of acetone. The reactions involved follow.



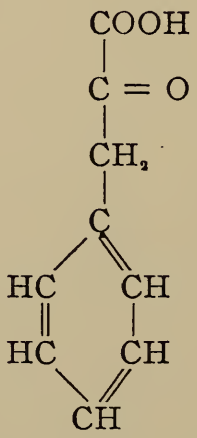
Leucine — Oxy-isobutyl acetic acid — Isovaleric acid — β-oxy-butyric acid

As a general rule substances containing the aromatic or benzene nucleus do not readily undergo complete oxidation in the organism, the benzene nucleus remaining unchanged. The amino acids derived from protein hydrolysis, and containing this nucleus, namely, tyrosine, phenylalanine and tryptophane do suffer complete disintegration, the benzene nucleus being disrupted. There are at least two ways in which the aromatic amino acids may be destroyed. In the first place the following series of reactions may occur—phenylalanine being employed as a specific example. Phenylalanine by oxidative deamination is first changed to phenyl-pyruvic acid:



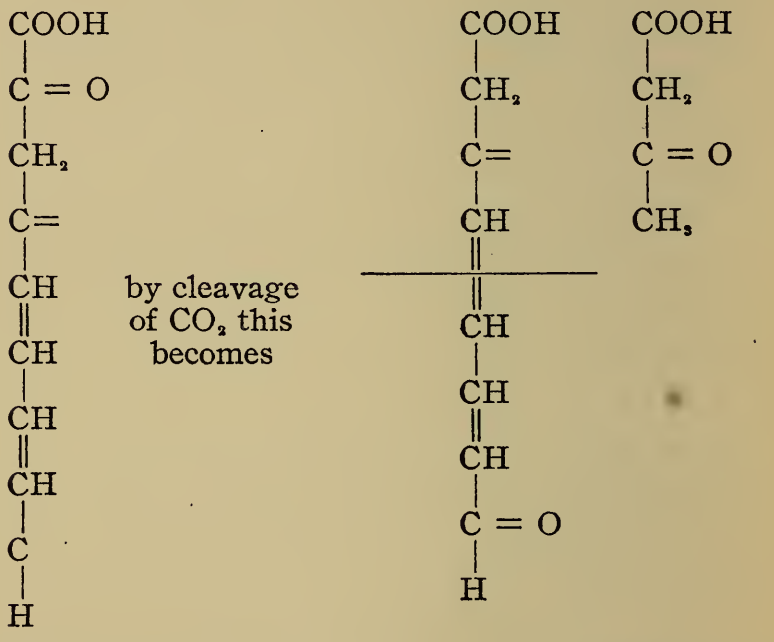
Phenylalanine

a ketone acid



Phenyl-pyruvic acid

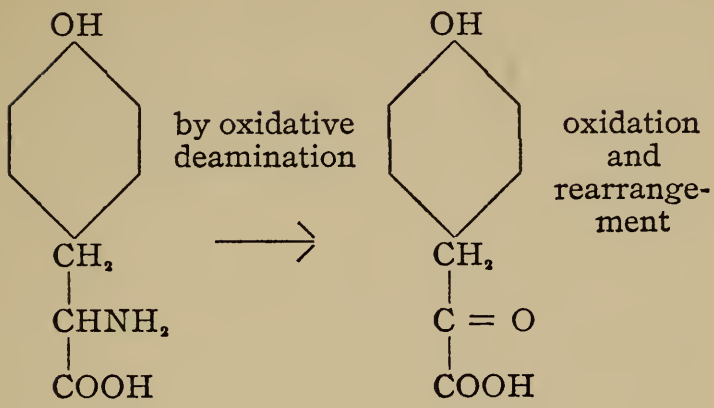
If we assume that the next step is the simple splitting open of the benzene nucleus of phenyl-pyruvic acid its formula may be written as follows:



Phenyl-pyruvic acid written in open chain form

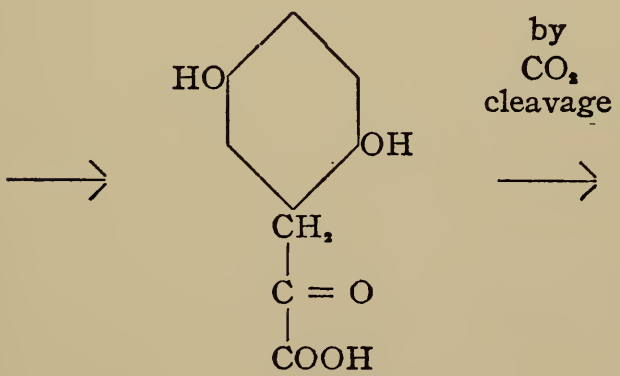
Diacetic acid

By the splitting in two of this chain and oxidation aceto-acetic or diacetic acid is produced which in turn may be directly oxidized to CO_2 and H_2O. Secondly, employing tyrosine as a specific example we may follow it through the following changes:

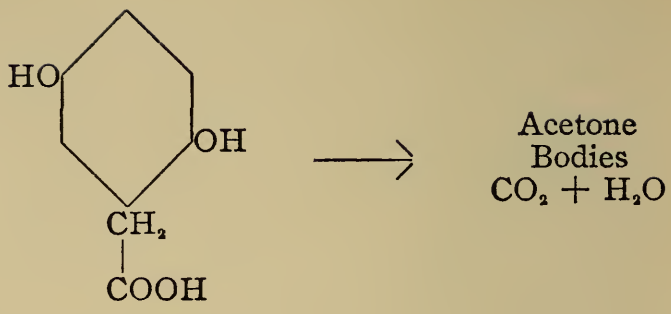


(Tyrosine) p.oxyphenyl amino-propionic acid

(Ketone acid) p.oxyphenyl pyroracemic acid



Hydroquinone pyroracemic acid

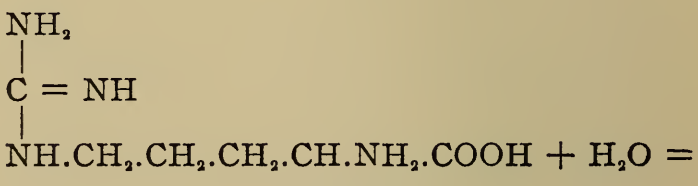


Homogentisic acid

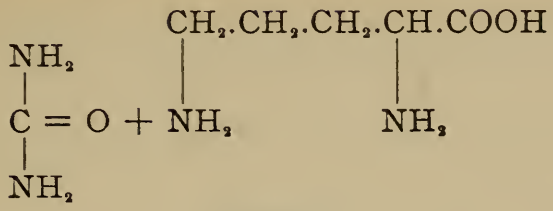
In these transformations homogentisic acid is an important intermediary product which at times appears in the urine. (See Alkaptonuria.) In harmony with the idea that homogentisic acid is an intermediary product in the decomposition of tyrosine and phenylalanine is the demonstration that the liver may form acetone from homogentisic acid.

Little definite is known concerning intermediary stages in the fate of trytophane in the human body.

Arginine undoubtedly undergoes hydrolysis by the enzyme arginase yielding urea and ornithine and the latter may also yield urea.



Arginine

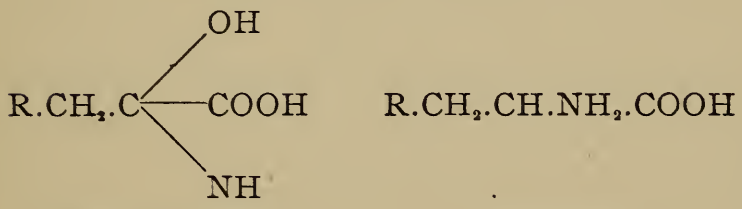


Ornithine

Synthesis of Amino Acids

It has been demonstrated that alanine, phenylalanine and tyrosine may be synthesized in the liver by perfusion of ammonium salts of ketone acids.

Ammonium salt of ketonic acid



Imino acid hydrate Amino acid

The possibility of synthesis of amino acids in this manner renders the interpretation of metabolic changes in the tissues more complex than ever and confers upon the organism a range of synthetic powers practically unlimited.

Under ordinary circumstances, however, it is unlikely that amino acids are synthesized to a great extent

in this way. The question has been tested experimentally in an indirect manner. If protein with a certain amino acid lacking is fed to animals it is reasonable to assume that if nitrogenous equilibrium can be maintained a synthesis of the missing amino acid must have occurred. From experiments planned to test this hypothesis it has been shown that no amino acids with the exception of glycocoll are ordinarily formed by synthesis. For glycocoll the evidence is strongly indicative of synthesis. As a rule in the body there is about 5 per cent of glycocoll nitrogen in every 100 grams of protein nitrogen. It is well known that benzoic acid ingested is united with glycocoll to form hippuric acid—in other words, benzoic acid feeding robs the body of glycocoll. If benzoic acid is fed in sufficient quantities to exhaust the possible content of glycocoll preformed in the tissues, the continued formation of hippuric acid must be provided for by glycocoll newly formed or synthesized. Hippuric acid does continue to be formed under these circumstances and hence glycocoll must be synthesized. It is possible, of course, that glycocoll may be formed from the transformation of some other amino acid, as by cleavage of a long change amino acid. Another evidence in favor of the synthesis of glycocoll is the following—milk proteins are very poor in glycocoll, yet suckling animals are capable in a short time of building up in their bodies proteins which contain far more of this amino acid than can be accounted for by the ingestion of glycocoll yielded by the milk.

The Relationship Between Carbohydrates and Amino Acids

1. *The Formation of Carbohydrate from Amino Acids*

For a long time it has been accepted that carbohydrate may be formed from ingested protein. To determine the mechanism of this transformation many experiments have been carried through upon animals. In particular the formation of glycogen from ingested protein has been subjected to experimentation and although the consensus of opinion would indicate that protein may give rise to glycogen formation, the experimental conditions under which most of the investigations were made are not free from criticism. The evidence of clinical experience, with diabetes, where fat or carbohydrate ingestion cannot always be held responsible for the large amounts of sugar passing through the kidneys daily, points positively to protein as the source of the carbohydrate excreted. In agreement with this conception is the observation that the urinary nitrogen and sugar excretion in the pathological state mentioned run along parallel lines.

How may this sugar formation be explained? One may assume, for instance, that protein contains groups of a carbohydrate nature or groups closely allied to the carbohydrates. Although it must be accepted that certain proteins do contain carbohydrate groups, the possession of such groups by proteins is by no means universal, and, on the other hand, one is unwarranted in stating that any specific protein will not lead to

sugar production. If we take a typical protein as egg albumin, and then on the assumption that all the nitrogen present is eliminated from the body as urea, we find left over a large carbon residue, the so-called "carbon moiety," of the protein which may be regarded as material capable of being transformed into carbohydrate. This potential of carbohydrate forming material must gain access to the blood stream, hence to the tissues, in the form of amino acids, in accordance with the present-day view of the processes of metabolism. The problem under consideration, therefore, resolves itself in the question, "Are amino acids capable of being transformed into carbohydrates?" The most convincing work in this direction is that of Lusk and his pupils. They have administered to dogs rendered diabetic with phlorhizin various amino acids and have observed that some yield sugar whereas others fail to do so. In their experiments the relationship between the dextrose and nitrogen of the urine, the D : N ratio, was determined. Under suitable conditions this becomes a constant. The ingestion of sugar forming substances changes this constant and any change serves as an index to the quantity of sugar formed from a given amount of substance introduced. It was found that the N-free parts of glycocoll, alanine, aspartic acid, and glutamic acid, containing respectively two, three, four, and five carbon atoms may be either completely or partially transformed to dextrose. All of the glycocoll and all of the alanine were converted

into glucose, whereas three of the carbon atoms contained in aspartic and glutamic acids were so changed.

The stages through which these amino acids are carried have been outlined by Lusk. In the first place it is assumed that the initial change is a hydrolytic deamination whereby ammonia is formed and a hydroxy group is added to the denitrogenized amino acid. According to this view glycocoll would first be changed to glycolic acid, which on reduction would yield glycolic aldehyde, three molecules of which will form one molecule of dextrose. The chemical relationships are shown below:

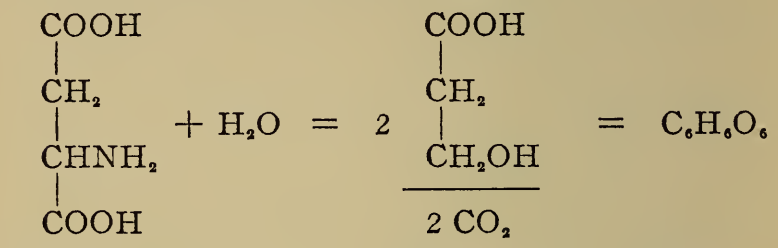
$$3\ \underset{\text{Glycocoll}}{\overset{\overset{CH_2.NH_2}{|}}{COOH}} + H_2O = 3\ \underset{\text{Glycolic acid}}{\overset{\overset{CH_2.OH}{|}}{COOH}} - 3\ O =$$

$$3\ \underset{\text{Glycolic aldehyde}}{\overset{\overset{CH_2OH}{|}}{CHO}} = \underset{\text{Dextrose}}{C_6H_6O_6}$$

For alanine the changes undergone are a direct transformation into lactic acid which is well known to give rise to dextrose production.

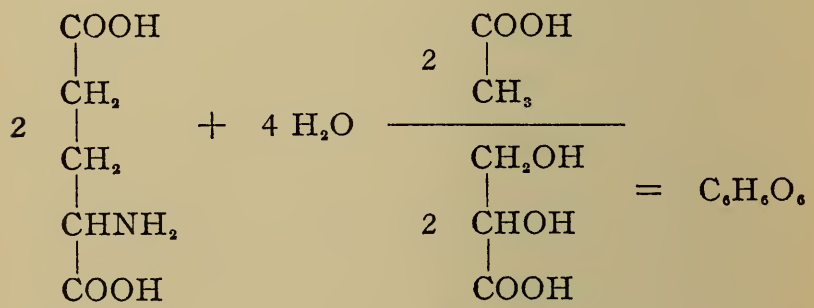
$$2\ \underset{\text{Alanine}}{\overset{\overset{CH_3}{|}}{\underset{|}{CHNH_2}}{COOH}} + H_2O = 2\ \underset{\text{Lactic acid}}{\overset{\overset{CH_3}{|}}{\underset{|}{CHOH}}{COOH}} = \underset{\text{Dextrose}}{C_6H_6O_6}$$

With aspartic acid only three of the carbon atoms are available for dextrose formation, the remaining carbon atom being changed to carbon dioxide in accordance with the following reactions:



Aspartic acid — β lactic acid — Dextrose

As with aspartic acid so also with glutamic acid only three of the carbon atoms are changed to dextrose, the two remaining carbon atoms being liberated in the form of acetic acid.



Glutamic acid — Acetic acid and Glyceric acid — Dextrose

Dakin has demonstrated that serine, proline, ornithine and arginine are all capable of yielding large amounts of sugar when given to glycosuric dogs.

Apparently arginine is the only amino acid with more than five carbon atoms which furnishes glucose freely. In this case it is probable that the sugar comes from the ornithine moiety with five carbon atoms, into which it may be converted by the action of arginase. Lysine is the only straight chain amino acid derivative of protein which fails to yield sugar. Although the relationship of the remaining amino acids to carbohydrate metabolism is less definitely established, Lusk has made the interesting calculation that in diabetes sugar may arise from protein to the extent of nearly 60 per cent. He says, "It becomes evident that there may be a condition of nutrition in which protein is used neither for repair nor for growth, but simply to be diaminized and subsequently to act like fat or carbohydrate as nutritive materials for the organism."

2. *The Formation of Amino Acids from Carbohydrates*

The formation of amino acids from carbohydrate material is a reaction less well known than the reverse process. The close relationship existing between lactic acid and carbohydrates on the one hand and lactic acid and alanine on the other suggests the ready transformation of glycogen to alanine presumably with lactic acid and ammonium pyruvate as intermediary products. With this suggestion in mind Embden perfused a liver rich in glycogen and found that alanine was formed. When, however, perfusion of a glycogen-

free liver was carried through alanine was not present in significant quantities. From experiments of this nature it is evident that the metabolic processes concerned in protein metabolism are intimately associated with those of the intermediary metabolism of carbohydrate, and further that at times at least protein may serve for both sources of nitrogen and carbonaceous material. Protein, therefore, should not be regarded in the strict sense merely as a purveyor of the nitrogen which is essential for life processes. It is much more than that, as has been demonstrated.

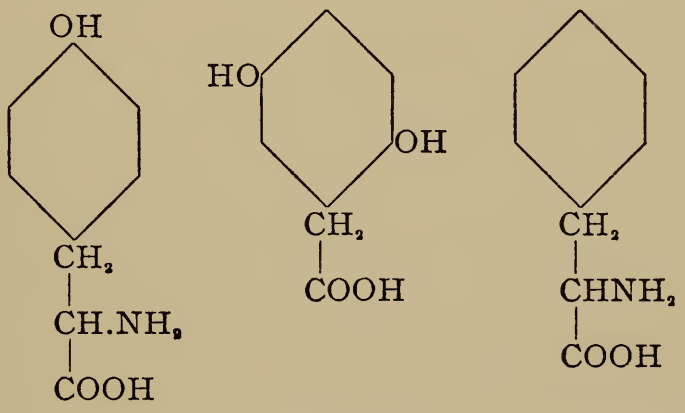
Anomalies of Amino Acid Metabolism

Alkaptonuria

In previous pages it has been pointed out that the normal organism is capable of demolishing the benzene ring as found in tyrosine and phenylalanine for under normal conditions no evidence of these substances can be found in the urine. For the successive steps assumed to occur in this destruction see p. 105. However, there are certain individuals who apparently are unable to break open the aromatic ring and in the urine is found an intermediary decomposition product, homogentisic acid. Urine containing homogentisic acid exhibits a tendency to turn dark on exposure to the air and may show a strong reducing action. This condition, known as alkaptonuria, is of rare occurrence and may last through life without affecting the health of the individual. It can scarcely be regarded as of

pathological nature, but should be looked upon rather as an anomaly of metabolism and is generally considered as being hereditary in origin. It occurs oftener in man than in woman and blood relationship, as first cousins, predisposes to the condition.

Whenever homogentisic acid is present in the urine, it is there in relatively large amounts for the anomaly is an absolute one, that is, apparently homogentisic acid formed is destroyed by the normal organism. The relationship of tyrosine phenylalanine and homogentisic acid are shown below:



Tyrosine Homogentisic acid Phenylalanine

The significance of alkaptonuria in connection with the metabolism of the amino acids is that the appearance of homogentisic in the urine of alkaptonurics gave the first hint as to the probable transformations occurring in the demolition of the benzene radical found in tyrosine and phenylalanine. That homogen-

tisic acid is a step in the degradations of these amino acids has been doubted by Dakin who believes that there is in these subjects an abnormal formation of homogentisic acid as well as an inability to destroy it once formed. In accord with this idea he has shown how tyrosine and phenylalanine may be destroyed without homogentisic acid as an intermediary product (see p. 103). On the other hand, it has been demonstrated by Abderhalden that normal individuals may eliminate homogentisic acid when excessive quantities of tyrosine are fed. Also the administration of tyrosine or phenylalanine, or of foods rich in tyrosine, that is proteins, causes a significant increase in the excretion of homogentisic acid. If alkaptonurics live on a protein-free diet for short periods of time the excretion of homogentisic acid is markedly diminished, but does not disappear entirely. Undoubtedly the aromatic amino acids formed from tissue metabolism do not suffer destruction to any greater extent than those introduced into the blood from the food protein. Why an alkaptonuric individual fails to destroy the tyrosine radicle is still a matter of conjecture.

Cystinuria and Diaminuria

Under ordinary circumstances cystine, the sulphur bearing amino acid fails to appear in the excreta, probably undergoing extensive destruction. The sulphur is oxidized to sulphuric acid and eliminated as a sulphate in the urine. In certain individuals, however, cystine appears in the urine and owing to its relatively

slight solubility is deposited as hexagonal crystals. It may also form cystine concretions in the bladder. The condition of cystinuria with that of alkaptonuria must be regarded as an anomaly of metabolism. Cystinuria appears to be a distinctly hereditary condition since it may appear in families for many generations, and apparently follows the Mendelian law of heredity. Like alkaptonuria it is found oftener in males than in females; it seems to lead to no pathological symptoms other than the formation of concretions. There is probably no complete failure to destroy cystine since only a portion of the cystine from protein ingested reappears in the urine. Undoubtedly a part of the cystine is catabolized in a normal manner. From the fact that cystinuria persists in the absence of protein intake and further that cystine fed to cystinurics fails to appear in the urine, the conclusion may be reached that the urinary cystine has its origin in that formed during catabolism of the tissues. It may be possible that in these subjects there is only a limited capacity for destroying amino acids in general for, in addition to cystine, leucine and tyrosine have been found in some cases.

At times the diamines cadaverine and putrescine formed by putrefaction in the intestine may also be present in the urine of cystinurics. The diamines are significant in that they are two of the so-called ptomaines. Putrescine and cadaverine occur also in diseased conditions of the intestinal tract, thus they may be found in various infections, in cholera, dysen-

tery, gastro-enteritis, etc. Their origin in putrefaction of protein decomposition products together with their appearance in the urine of cystinurics led to the view that the cystine in the cases mentioned had a like source. This, however, has been shown to be incorrect. A second view was that diamines interfered with sulphur oxidation in the organism, hence the appearance of the unoxidized cystine. This idea has been shown to be untrue for cystinuria may occur in the absence of the diamines, and the administration of diamines has no influence upon the output of cystine in the urine. "In intestinal disturbances, it is probable that these compounds are the result of bacterial activity—indeed, they may be the metabolic end-products eliminated by bacteria. In cystinuria, however, it is possible that a different explanation for diaminuria is pertinent. It may be assumed, for instance, that in the beginning cystinuria and diaminuria are brought about through a similar, or indeed the same cause, or causes, for example, a gradually changing type of metabolism induced by some unknown agency, resulting in an anomaly of metabolism. If the anomaly is slight in character, cystine alone is eliminated as a result, whereas if the change in metabolism is sufficiently pronounced diamines are also excreted. If this assumption is accepted it is easy to explain why in some cases of cystinuria the diamines are absent, and that gradually one or both of these compounds disappear, that cystinuria persists, but that cystinuria does not cease and leave diaminuria."

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CHAPTER VII

THE AMINO ACIDS IN RELATION TO THE SPECIFIC DYNAMIC ACTION OF PROTEINS

The view has been held for many years that the ingestion of protein increases the power of the body cells to metabolize materials brought to them. More recently it has been shown by Rubner that under suitable conditions each foodstuff is capable of exerting a specific accelerating influence upon the energy metabolism. In order to maintain life Rubner believes that a fixed requirement of energy is necessary. When the organism is fasting, the essential energy requirement may be furnished from the tissues of the organism itself. After their ingestion the foodstuffs are changed in various ways with the evolution of heat until finally they are transformed into materials which are capable of supporting vital phenomena. The final products formed may be employed for the replacement of the substances oxidized during fasting. The heat produced in the formation of these compounds is added to the heat produced for the maintenance of vital processes, and the total heat production, therefore, exceeds that found in starvation. This increased

heat production induced by each foodstuff is different, and is specific for each foodstuff. Rubner, therefore, has named this effect "specific dynamic action."

It has been shown under the correct conditions that if the energy metabolism of a fasting dog be represented as 100 calories, food must be given in the following amounts to prevent body loss, 106 calories of sugar, or 114 calories of fat, or 140 calories of protein. In an experiment by Rubner it was found that a fasting man metabolized 2042 calories. After the ingestion of 2450 calories of sugar he metabolized 2087. When given 2450 calories of meat 2566 calories were metabolism. Whatever the cause of the greater metabolism of protein ingestion it is believed that there is produced a liberation of energy which cannot be used by the tissues in support of their activities but it is possible that it may contribute to the maintenance of body temperature. On a mixed diet this liberation of heat unavailable for energy purposes is not of great significance in the total metabolism since it increases the metabolism of energy less than one tenth on a maintenance diet above that when no food is eaten.

Another explanation for the increased energy metabolism after the ingestion of foodstuffs has been put forward by Zuntz, who ascribes this effect to the mechanical work of the intestinal canal (Darmarbeit) performed during digestion and assimilation. Such a theory would seem to fit in well with the greater specific dynamic action of protein and its probably greater difficulty of digestion in comparison with

sugar. To test the Zuntz hypothesis Benedict has attempted to produce the effects of food ingestion such as augmented mechanical work through increased peristalsis produced by large doses of purgatives, as sodium sulphate. In spite of greatly increased peristalsis the administration of sodium sulphate failed to show any measurable increase in metabolism. "In the belief that when the intestine is full of partly digested food products and epithelial debris, the amount of mechanical work thereby incurred might be greater than that involved in several powerful peristaltic waves, experiments were made in which relatively large amounts of agar-agar were ingested, thus producing a bulky, voluminous stool. The agar-agar being practically non-oxidizable, there was no great complication due to the combustion of carbohydrate from the agar-agar. With the agar-agar it is reasonable to assume that there must have been an extensive segmentation process as well as peristaltic waves. But even under these conditions on the ideally controlled experiments there was absolutely no increase in metabolism. In so far, then, as the experiments on men show with controlled conditions, the work of peristalsis and probably of segmentation is not sufficient to be measured in the great daily energy transformation of the body. It is impossible to think of muscular activity of any kind taking place without some slight increased metabolism, but the amount involved in intestinal activity must be so small as to

be entirely negligible in the extensive energy transformations for body maintenance."

These results are in accord with conclusions reached earlier by Rubner who denied that the work of digestion and assimilation could be held responsible for the effects observed after food ingestion.

Benedict is of the opinion that from the food substances are absorbed which, carried by the blood, stimulate cells to greater activity, and he further indicates that these unknown bodies are of an acid character. From his extensive investigation on the influence of amino acids upon metabolism, that is, their specific dynamic action, Lusk believes that the explanation of Rubner as to the cause of specific dynamic action must be revised. "Amino acids act as stimuli upon the cells, raising their power to metabolism. They may act instead of nerve stimuli when increased heat production is required in the presence of external cold—the chemical regulation of temperature of Rubner. The energy liberated in response to these stimuli may be supplied by carbohydrate or fat. When fat and carbohydrate are given separately or together there may be an increased heat production on account of the increase in the quantity of materials available for the nutrition of the cells. With the cessation of absorption and the return of the blood to the composition it possessed before food was taken the metabolism falls to its basal value." When glucose and an amino acid, as alanine, are given together the metabolism is increased to a point where the resultant effect

is nearly equal to the sum of the two individual influences. This indicates a distinct difference between the cause of the specific dynamic action of glucose and that of alanine. Two types of processes are here suggested, namely, a metabolism of plethora and amino acid stimulation. Carbohydrate or fat metabolites which are being absorbed from the intestine into the blood bring about a metabolism of plethora. In the metabolism of plethora the influx of carbohydrate or fat enables the cells to oxidize at a higher level through the increased mass action of food particles which are available. (Lusk.) A recent attempt by Lusk to explain "amino acid stimulation" of the cells has resulted in the conclusion that some at least of the amino acids even when they are not oxidized "yield products of metabolism, either hydroxy or ketone acids which act as stimuli to induce higher oxidation in the organism. This is the conclusive proof of a true chemical stimulation of protoplasm within the mammalian organism. It explains the specific dynamic action of protein."

As has been shown repeatedly throughout this book the effects characteristically produced by protein are gradually being ascribed as a function of the amino acids. The amino acids therefore may be regarded not alone as pabulum for the restoration of depleted cells but must also be looked upon as playing a distinct and significant rôle in the rate or extent of cellular metabolism.

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CHAPTER VIII

THE AMINO ACIDS AND SIMPLER NITROGENOUS COMPOUNDS AS FOODSTUFFS

Value of Amino Acids as Foodstuffs

The dictum of Liebig that the animal organism is endowed with very limited capacities for processes of synthesis was accepted for a great many years without serious question. A partial reason for this assumption is to be found in the great difficulties to be overcome in putting the question to experimental proof. The discovery of erepsin by Cohnheim with the consequent readjustment of ideas relative to the extent and character of digestion processes and the form of products absorbed casts doubt upon the inability of the animal body to synthesize. It was reasonable to assume that the disintegration of the protein molecule to the stage of amino acids is with a purposeful object and that the absorption of such relatively small compounds as amino acids predicates the probability that synthesis must occur if the organism is obliged to reconstruct new protoplasm to replace that worn away through the many metabolic activities.

About a dozen years ago the first attempt to determine the possibility of positive protein synthesis was

made by Loewi. This investigator allowed protein to digest until it no longer gave a reaction with the biuret test an indication that products possessed of a protein nature had all been reduced to a lower stage. Upon feeding this mixture of amino acids together with fat and carbohydrate to a dog, Loewi demonstrated that nitrogen in the form contained in his digestion mixture was not alone capable of maintaining the life of the animal, but furthermore kept it in a state of nitrogenous equilibrium, a retention of nitrogen together with an increase in weight being observed. The experiments of Loewi were quickly followed by those of Henderson and Dean, who were the first to employ digestion products formed through the action of acids rather than by ferments. They found nitrogen retention but were uncertain whether it signified protein synthesis. As a result of many succeeding investigations it soon became clear that the power of the digestion products to replace body tissue depended upon the manner in which such products were formed. To put it differently, digestion products formed from protein by the agency of enzymes were fully capable of supplying to the body its necessary quota of nitrogen whereas those products obtained from proteins through the action of acids could not take over so completely this function but were regarded as of value inasmuch as they could be looked upon as being "protein sparers." In this connection it may be well to cite some experiments of Abderhalden and Rona with mice. To these mice were fed different prepara-

tions of casein together with sugar. To one series of mice unchanged casein was fed, to a second, casein that had been digested with pancreatin for a period of two months, a third series received casein digested for one month with pepsin-hydrochloric acid and then for two months with pancreatin, the fourth series were fed with casein hydrolyzed for ten hours with 25 per cent sulphuric acid. The results showed that those animals fed with casein digested with pancreatin for two months and those given unchanged casein lived about the same length of time. Mice fed on the other two preparations lived shorter periods of time. Other investigators obtained similar results. An interesting controversy now arose as to the reason for the specific difference between products formed by enzymes and those resulting from acid hydrolysis. Abderhalden and Rona from their work cited above put forth the hypothesis that the difference in the two products lay in their content of polypeptides. According to this view digestion by ferments results in the presence of considerable amounts of fairly complex polypeptides which serve as nuclei for the synthesis of new protein material. Hydrolysis by acid, however, carries the digestion beyond the stage of polypeptides, hence, no nuclei for synthesis are present and the inability of acid digestion mixtures to fully serve as nitrogenous pabulum is explained. In support of their hypothesis they offer the observation that the casein preparation formed by pancreatin action contained only 16 per cent of polypeptides, that of the

pepsin-hydrochloric acid mixture further subjected to the influence of pancreatin contained only half as much polypeptides, whereas from that formed by acid hydrolysis polypeptides were entirely absent. Later, however, it was shown by Abderhalden and his co-workers that the varying content of polypeptides cannot be the sole reason for the differences observed in the two classes of products in their ability to supply the nitrogenous needs of the body, for a dog was kept alive for thirty-eight days and the only supply of nitrogen was in a digestion mixture containing only amino acids. Again, a young dog gained weight and retained nitrogen in completely digested meat and a bitch was kept in nitrogenous equilibrium during lactation with meat digested to the amino acid stage. Abderhalden and London were able to maintain a dog with an Eck fistula (the liver shunted out of the portal circulation) on fully digested meat. From this experiment they further concluded that the liver could play a small rôle only in protein synthesis and used these results as support for their view that protein synthesis occurs during absorption.

From the work of Henriques, Abderhalden and others it soon became evident that the difference in nutritive value between ferment and acid hydrolysis products could not be ascribed wholly to the presence or absence of polypeptides. Upon closer investigation it developed that the failure of acid hydrolytic products to meet nutritive requirements satisfactorily could be explained by the fact that during acid hydroly-

sis tryptophane, unquestionably one of the most important of the amino acids, is destroyed. The proof that herein lies the true explanation was furnished by Abderhalden and Frank, who succeeded in maintaining dogs in nitrogenous equilibrium on meat completely hydrolyzed by acid to which had been added a small amount of tryptophane.

After the demonstration that the nitrogenous nutritive requirements of the organism can be supplied by a mixture consisting of the products of hydrolysis, whether by acids or enzymes, an attempt was made by Abderhalden to support a dog in nitrogen equilibrium on an artificial mixture of amino acids to which were added carbohydrate and fat. The successful outcome of the investigation led Abderhalden to declare that the animal organism is capable of forming all the tissue constituents out of the simplest derivatives of the proteins. Inasmuch as carbohydrate and fat may be prepared synthetically, as may some of the amino acids, the problem of the artificial production of foodstuffs is solved according to Abderhalden, who says that such a possibility is limited only by the question of sufficient funds. From these observations it becomes evident that mixtures of amino acids are fully capable of supplying the nitrogenous needs of the organism when applied to the lower animals. An opportunity was afforded Abderhalden and his coworkers to extend this type of investigation to man. A boy with a stricture of the œsophagus on whom gastrotomy had been performed was the subject. To

him was given per rectum a mixture of protein (meat) digestion products obtained through the combined action of trypsin and erepsin. The experiment was continued for fifteen days and during this period nitrogen equilibrium was maintained, the body weight increased and the general condition of the subject was excellent.

This brief review of the salient features of the problem leads to but one conclusion, namely, that the amino acids must be regarded as foodstuffs capable of supplying the nitrogenous needs of the organism, and that the chief factors to be taken into account with regard to the nutritive value of any protein or proteins are the character and the extent of the amino acids contained therein.

The Value of Amides and Ammonium Salts as Foodstuffs

The nutritive value of various simple nitrogenous compounds has been a subject for investigation for many years. This is especially true for such substances as the amides and has been of particular interest to those concerned with agricultural problems since in the food of herbivora amides may play an important rôle. From the viewpoint of nutrition in general, the proof that animals may thrive on amides or other simple nitrogenous compounds supplied as sources of nitrogen carries with it indirect evidence of

the transformation of these substances into amino acids—in other words, amino acid synthesis occurs.

Particular attention has been paid to the determination of the value of asparagine as a source of nitrogen, one of the first investigators being Mercadente, who believed that protein formation could take place from asparagine, especially in plants. Sachse believed that protein was formed from asparagine by the simple addition of fatty aldehydes. On the other hand, Loewi thought that in the presence of carbohydrates protein was formed from asparagine by a series of condensations. Zuntz suggested that in herbivora asparagine was built up into protein by bacteria in the intestine previous to utilization. This latter view has been supported by numerous investigators, some of whom state that protein-forming bacteria are widely distributed in nature and may act very efficiently and quickly when in suitable environment. The evidence available seems to speak strongly in favor of the view that asparagine may serve as source of nitrogen or act at least as a protein sparer, as much as two-thirds of the protein in the diet of herbivora being replaceable by asparagine. On the other hand, several investigators claim that asparagine cannot take the place of protein at all, hence, cannot be used as a source of nitrogen and that even the degree of protein sparing action that may be exhibited by this amide is extremely limited.

In a comparable manner it has been suggested that various ammonium salts may also replace protein to

a certain extent at least, or act as protein sparers. The problem of the utilization of ammonium salts subjected to much experimentation in the past has recently been revived through the work of Grafe and Schläpfer who have asserted that ammonium salts, urea, and even nitrates may serve as sources of nitrogen for the animal organism, and they regard the utilization as indicated by nitrogen retention as a process of amino acid synthesis. These results have been assailed by others on the ground that the observed retention of nitrogen as a result of feeding the above-mentioned compounds may be explained in other ways than as a proof of amino acid synthesis. "There are several ways in which they may be assumed to behave in the organism. In the first and foremost instance they may serve as pabulum for the alimentary bacteria, which in turn are destroyed in large numbers in the digestive tract and can furnish a yield of perfect protein synthesized from simple compounds like urea and the salts of ammonia. It is generally admitted that in certain species like the herbivora, in which bacterial processes have a free play in the gastro-intestinal tube, the contribution of dead bacterial bodies to the intake is by no means negligible." "The feeding of urea or ammonium salts might lead to an apparent nutritive advantage by depressing or inhibiting the usual breaking down of nitrogenous compounds in metabolism. This would accord with the belief that the products of cellular waste themselves tend to impede cellular metabolism. Now that

the synthesis of amino acids from ammonia and carbohydrates has been accomplished directly or indirectly in the laboratory, the possibility of a similar reaction in the body must be reckoned with. Finally, the alleged utilization of urea and other simple nitrogen derivatives may merely be an instance of unsuspected retention and delayed excretion. Even so soluble a salt as an iodide may not be entirely recovered in the excreta until several days after its administration has been stopped. Surely no one would look on the temporary deficit as an indication of nutritive 'utilization' of the foreign salt."

Various possibilities therefore present themselves in the interpretation of the alleged utilization of these simple nitrogenous substances. The influence of alimentary bacteria may be eliminated by parenteral feeding of the compounds, which, however, has not been feasible until recently when Henriques succeeded in devising a method whereby a slow constant stream of nutritive solution may be intravenously introduced into the body. Subjecting utilization of urea and ammonium salts to the test by means of this device, Henriques and Anderson have demonstrated that no permanent retention of these nitrogenous compounds occurred. It is therefore exceedingly improbable that the body itself is in a position to transform these substances into amino acids. Amino acid synthesis is not an easy task for the organism nor is there evidence that even the transformation of one amino acid to another is accomplished to any extent. The organism

must have ready formed amino acids supplied to it in sufficient quantity and variety if it is to accomplish its task of tissue building.

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CHAPTER IX

THE SPECIFIC ROLE OF AMINO ACIDS IN NUTRITION AND GROWTH

That the chemical differences in proteins as determined by their amino acid content must be of considerable significance in metabolic processes has been understood in a vague way for a long time. As soon as recognition was gained for the view that the problems of nutrition are concerned with other factors than a mere sufficiency of nitrogen or an adequate intake of potential energy the problems of intermediary metabolism forced themselves upon the attention of physiologists and led to a thorough appreciation of the value in nutrition of factors previously entirely overlooked or considered of little or no moment.

Reference to the table on p. 22 will bring out clearly the differences that exist between a few of the typical proteins. The most striking variations in amino acids from a *quantitative* viewpoint are evident. Such differences are undoubtedly of importance from a nutritional standpoint, but of much greater significance are the qualitative variations. To point out briefly the most evident of these it may be seen that

albumin and casein are glycocoll-free. Gliadin from wheat contains no glycocoll and only a trace of lysine. Zein from maize yields no lysine nor tryptophane and gelatine contains no cystine, tyrosine nor tryptophane.

The first appreciation that qualitative differences in protein composition may be of importance in nutrition was furnished by the classic experiments of Voit and Munk, who showed that gelatin could not support nitrogen equilibrium. The demonstration by Escher that the addition of tyrosine improved the powers of gelatin in establishing nitrogenous equilibrium gave rise to a series of investigations, the results of which have led to a much more complete understanding of the problems intimately connected with metabolism. Only a few of these, however, need be reviewed here. Kaufman was able to show that when gelatin is fed to man and dogs with the addition of the missing amino acids, tyrosine and tryptophane, nitrogen equilibrium could be maintained for short periods at least. The work of Willcock and Hopkins with zein, which it will be remembered is deficient in lysine and tryptophane, is of great interest in the present discussion as it attacked the problem from new viewpoints, in entire accord with the conceptions of the present. In their introduction these authors point out that: "We are no longer bound to Liebig's view, or to any modification of it which implies that the whole of the protein consumed is utilized as intact protein: nor are we even compelled to assume that the whole of what is broken down in the gut is resynthesized before

utilization. Protein products may function in other ways than in the repair of tissues or in supplying energy. It is highly probable that the organism uses them, in part, for more specific and more immediate needs. The discovery of substances absolutely essential to life, highly specific, and elaborated in special organs, suggests that some part, at least, of the protein products set free in the gut may be used directly by these organs as precursors of such specific substances. In adrenaline, for instance, we have an aromatic substance absolutely essential for the maintenance of life, and it is probable that the suprarenal gland requires a constant supply of some one of the aromatic groups of the protein molecule to serve as an indispensable basis for the elaboration of adrenaline. If this be so, it is certain that the gland itself could not, in starving animals, supply sufficient of such a precursor to outlast the observed survival periods. Since adrenaline must be produced at all costs, the required precursor must, in starvation, be obtained by tissue breakdown outside the gland. We may be sure, however, that adrenaline is far from being the only substance elaborated to which such considerations apply. Similarly, in an animal upon a diet sufficient to supply energy, but lacking in some essential group, the minimal waste in the general tissues of the body will be determined by the special need of the organs for the missing group. On this basis we have a hypothesis to account for the special protein-sparing properties of gelatin. It shares with protein certain

molecular groupings needed to satisfy specific needs, and is thus superior to fats and carbohydrates as a protein-sparer: it lacks, on the other hand, certain necessary groupings, fails therefore to supply all such needs, and thus cannot replace protein."

These considerations served as the basis for the experiments described by Willcock and Hopkins. Mice kept under exactly similar conditions were fed with a diet having zein as its source of nitrogen. In certain instances small quantities of tyrosine or tryptophane were added to the dietary. The results of the influence of such diets were measured by the "survival period"—that is, the period necessary to cause the death of the animal. In Fig. 1 is reproduced a diagram illustrating very clearly the influence of tryptophane upon the survival period. With zein as the only nitrogenous component of the diet young mice were shown to be unable to maintain growth. Tryptophane addition does not make zein capable of maintaining growth, but does greatly prolong the survival period. In Fig. 1 the survival periods of mice fed upon zein alone are not given, for they were identical with those obtained with mice fed zein plus tyrosine. Although added tyrosine exerted no influence upon the survival period, it must not be inferred that this amino acid is without specific effect on metabolism: it evidently played little rôle here because zein fed supplied sufficient tyrosine, hence an excess was without special influence. In reality tyrosine was added as a control to tryptophane addition, in order to determine

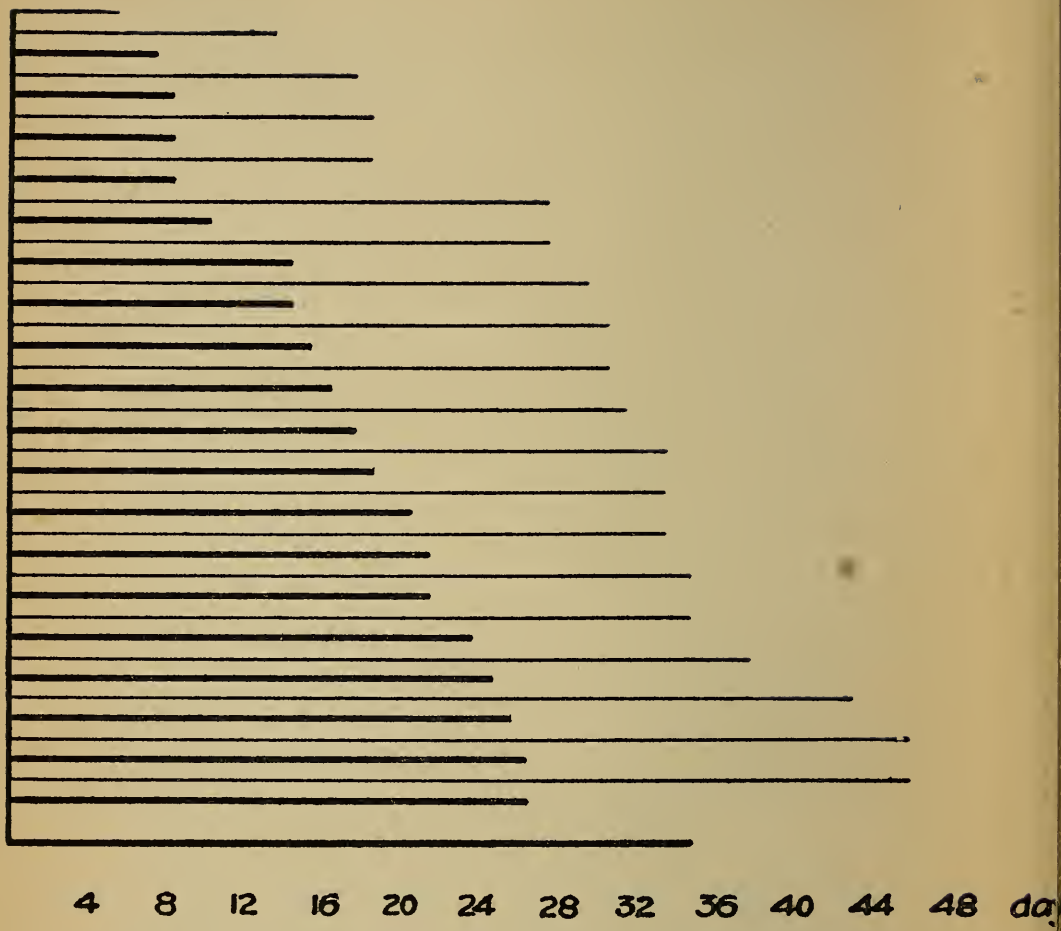


FIGURE 1. The thick lines show the survival periods (in days) of twenty-o individual mice upon the zein diet with tyrosine added. The thin lines show t same for nineteen mice upon the zein diet with tryptophane added. [From t *Journal of Physiology*, volume 35.]

whether addition of *any* amino acid would produce an effect, and hence, therefore, to find out directly the specific action of tryptophane.

A prominent feature in connection with the mice given zein alone was a condition of torpor; the mice were very inactive and made no movement when handled or touched, the ears, feet, and tail were cold, the coat was glairy and the eyes were half-closed. Those fed tryptophane with zein showed a strikingly different behavior, being active and apparently healthy even up to the end of life. In both instances death was not caused by a lack of food intake, as all animals gave evidence of appetite. Quantitatively, sufficient food was received but qualitatively something essential to life was lacking. It is possible that had lysine, the other amino acid lacking in zein, been fed also, even better results would have been obtained. Tryptophane undoubtedly is essential for the maintenance of life, although the specific rôle it plays has not yet been determined. As the authors mentioned above point out, "If it [tryptophane] serves as a basis for the elaboration of a substance absolutely necessary for life—something, for instance, of an importance equal to that of adrenaline—then, in starvation, or when it is absent from the diet, a supply is likely to be maintained from the tissue-proteins, the demand for it would become one of the factors determining tissue breakdown. In the case of young animals which directly benefit from the addition of a protein constituent otherwise absent from their diet, to the extent

of a well-nigh doubled life, and lose, instead of gaining, weight, the utilization of the constituent would seem to be of some direct and specific nature." These words give the first definite suggestion that individual amino acids may play a specific rôle in the maintenance of nutritional rhythm.

The failure of zein as a suitable source for the essential nitrogen requirement leads to the query whether any single protein will suffice in this respect. Attempts to answer this question have been many and it is only recently that a satisfactory positive reply has been given. In many of the older experiments lack of success has been attributed to various factors other than the character of the protein, and where apparently successful results have been obtained criticism has been pertinent in that, in most instances, the protein or proteins employed have not been free from impurities. The general impression gained from this type of investigation has been that sooner or later animals die when kept for a prolonged period upon a constant diet even though an abundance of energy producing material may be present. A so-called "pure" diet has been deemed impracticable. Lunin, one of the early investigators of the problem, fed mice with mixtures of casein, fat, cane sugar, and milk ash. On this artificial diet death occurred in from twenty to thirty days, a survival period greater than when the ash of milk was omitted. Mice fed dried milk were alive at the end of two months. Hall with mice and Steinitz with dogs obtained comparable

results when a similar form of dietary was used. By considerable variation in the non-nitrogenous portion of the food Röhmann showed that mice will thrive for weeks. A criticism of these experiments is that the range of variation in the make-up of the dietary resulted really in furnishing the animals an ordinary mixed diet. The experiments of Jacob with pigeons, of Falta and Noeggerath, and of Knapp with rats demonstrated that variety in the dietary undoubtedly tends toward prolongation of life but that death eventually ensues.

After experiencing many failures, Osborne and Mendel have succeeded in maintaining white rats for long periods of time upon single, pure, isolated proteins, growth also being at a normal rate. They attributed their success to the addition to the dietary of what they term "protein-free milk." This is prepared by removing the protein and fat from milk, leaving the milk sugar, inorganic salts and the unknown components. "Protein-free milk" always contains very small quantities of protein but not enough to support life. They have also demonstrated that by artificially imitating the composition of "protein-free milk" by union of the various ions fairly successful results have been obtained. It is therefore possible to construct a dietary in such a manner from purified isolated foodstuffs and artificial salt mixtures that animals may not only be maintained but normal growth may also be induced.

In their work, Osborne and Mendel differentiate

sharply between a maintenance diet and one capable of promoting growth. They have shown, for example, that a young animal may be maintained on a certain diet indefinitely without manifesting any tendency to grow. From the work of Donaldson it has been demonstrated that the life span of the white rat is about three years. Sexual maturity is reached in sixty days. The first year of life for the rat corresponds to the first thirty years of human life, and the curve of growth for this period is reproduced below. Fig. 2.

As an illustration of the influence of an isolated protein, casein (fed with starch, sugar, agar, lard, and a salt mixture), the chart, Fig. 3, is shown. It is evident that casein as the sole source of nitrogen was apparently incapable of allowing normal growth in a young rat during a period of forty-six days. In other words, stunting occurred. In period 2, casein and sugar were replaced by milk. Growth was resumed. The influence of changing the salt mixture content of the food intake is quite evident in periods 3, 4, and 5. The ability of milk to furnish the necessary nitrogen requirement is well shown in the chart, Fig. 4, the curve obtained being to all intents and purposes identical with the normal growth curve.

If to the casein diet "protein-free milk" is added, instead of whole milk replacing casein, normal conditions obtain as is well illustrated in the chart, Fig. 5.

Casein alone was found to be unable to support growth. In Fig. 6 is shown a curve in which, during period 2, casein was the only source of protein and

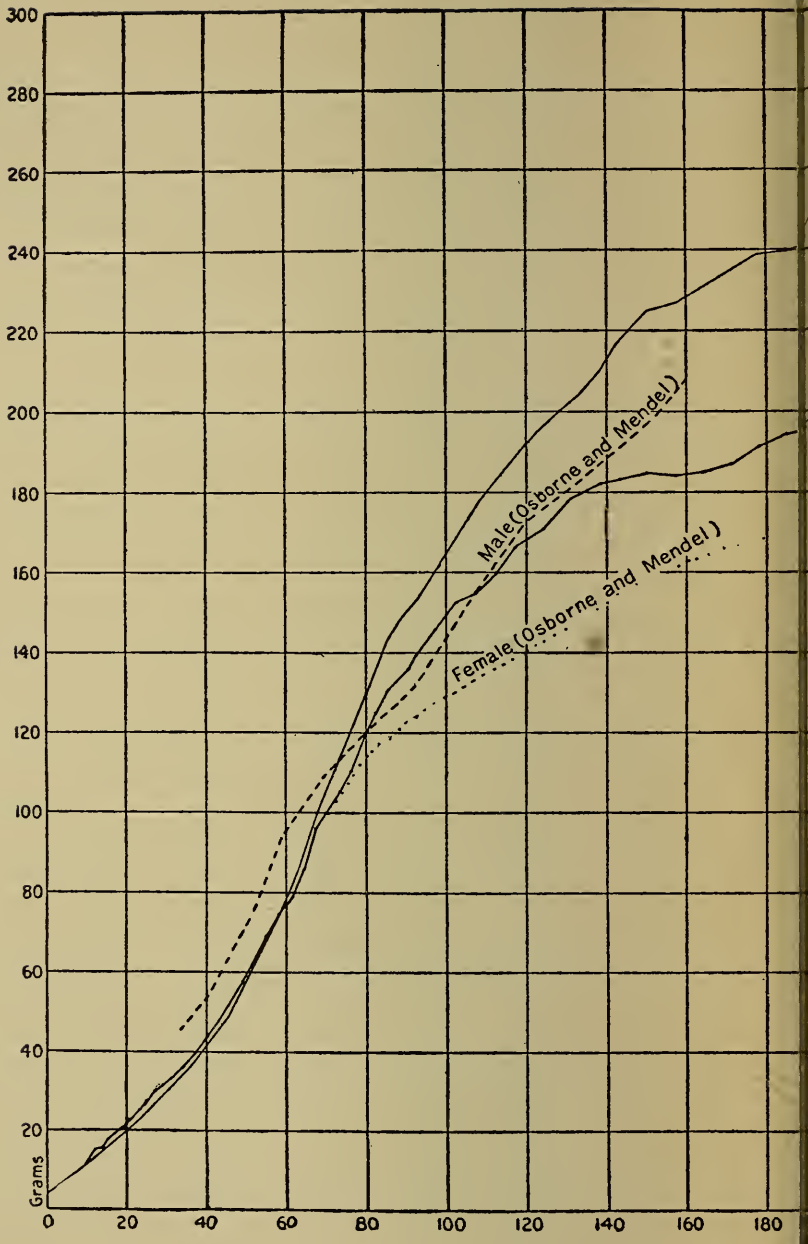
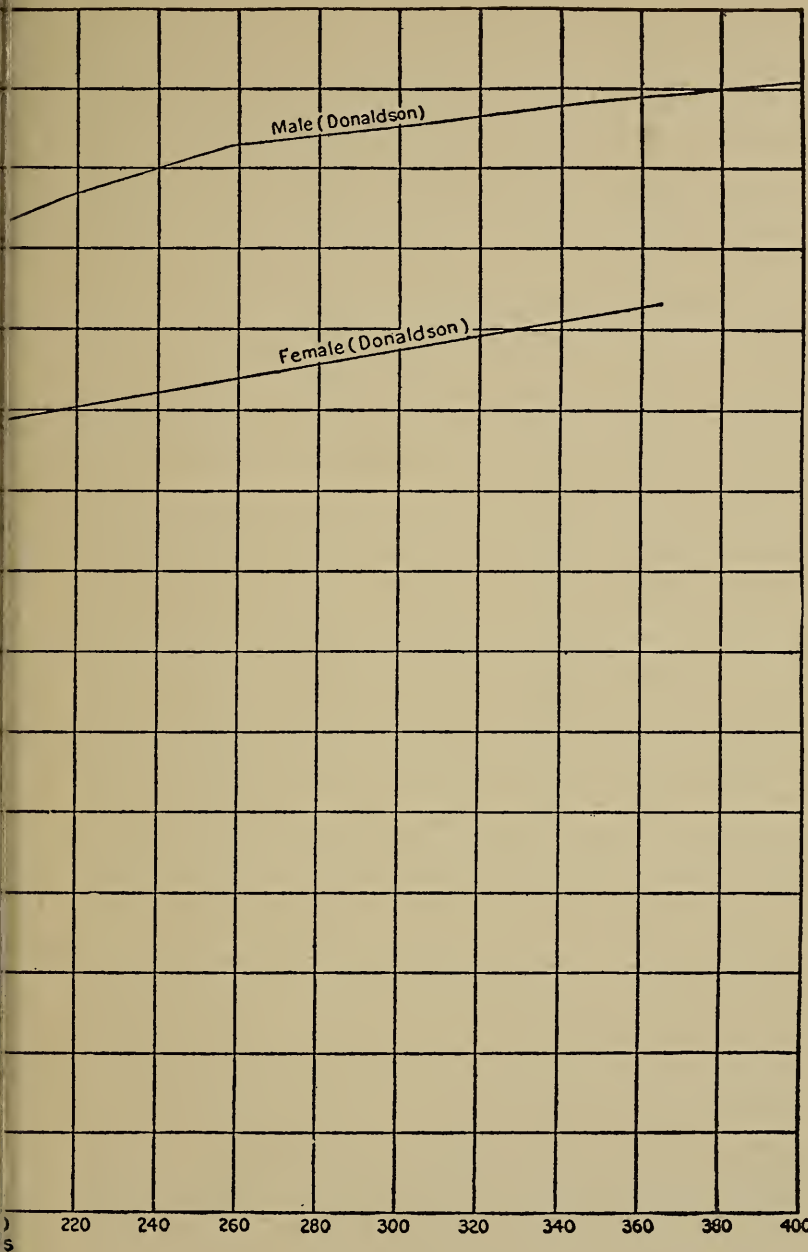


FIGURE 2 shows average normal rates of growth of male
an



female white rats according to Donaldson and to Osborne
ndel.

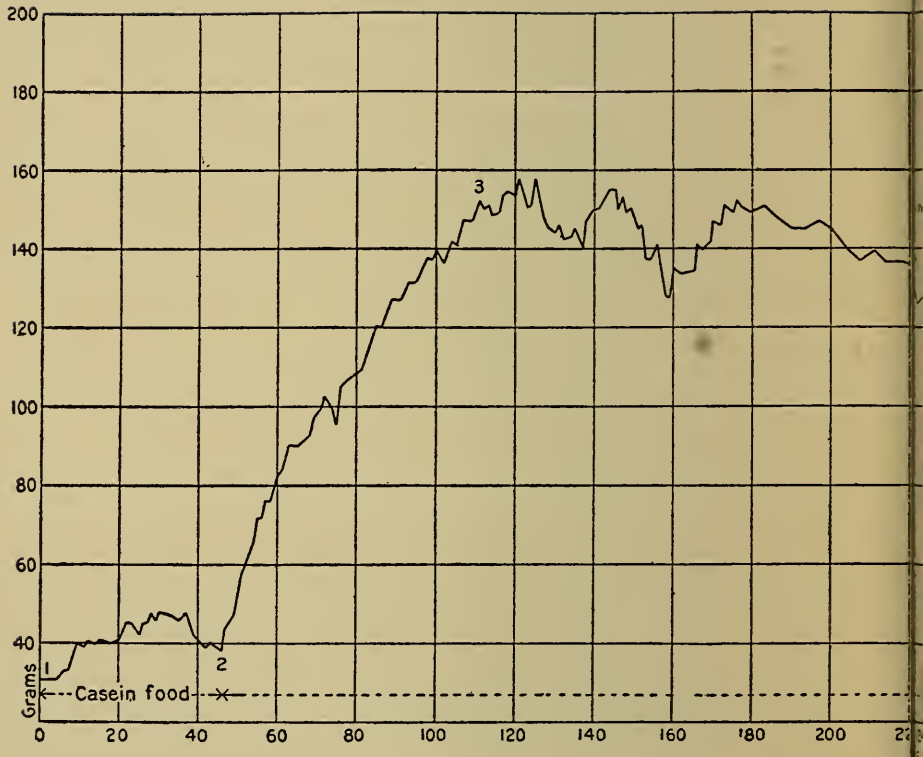
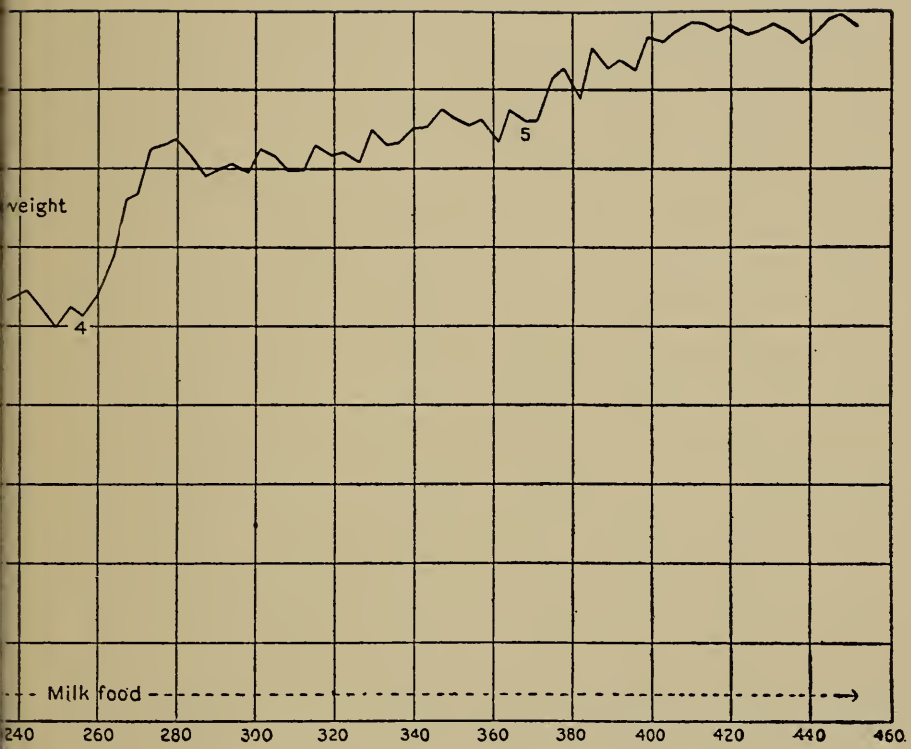


FIGURE 3. GROWTH CURVE



H Casein and Milk Diets.

as a result a decline set in, which could not be checked by doubling the percentage of casein in the diet. That lack of protein can not account for the decline is well shown in period 4, during which the original amount

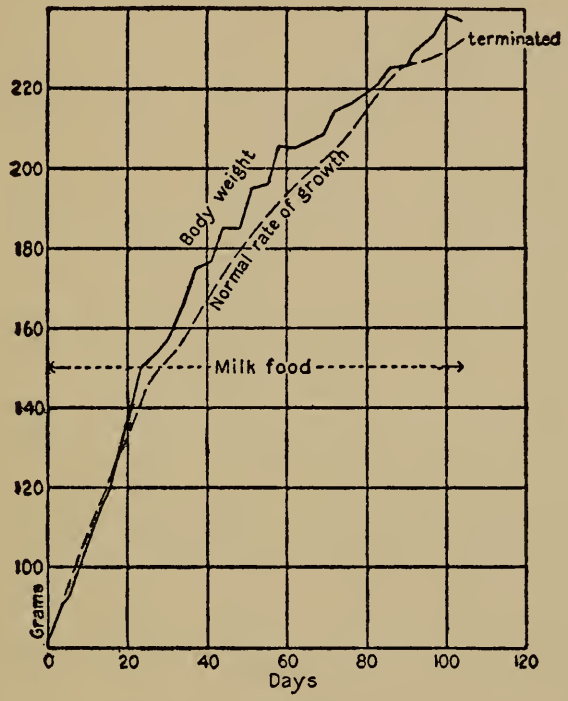


FIGURE 4. GROWTH CURVE WITH MILK DIET.

of casein was replaced and "protein-free milk" was also added. An immediate response in appetite was evidenced and speedy recuperation and growth were in order. This experiment demonstrates that a rat unable to maintain itself on an isolated protein may be caused

to speedily resume a normal condition by the addition to the diet of "protein-free milk."

From these and many similar results it is apparent that if suitable non-protein constituents of the dietary

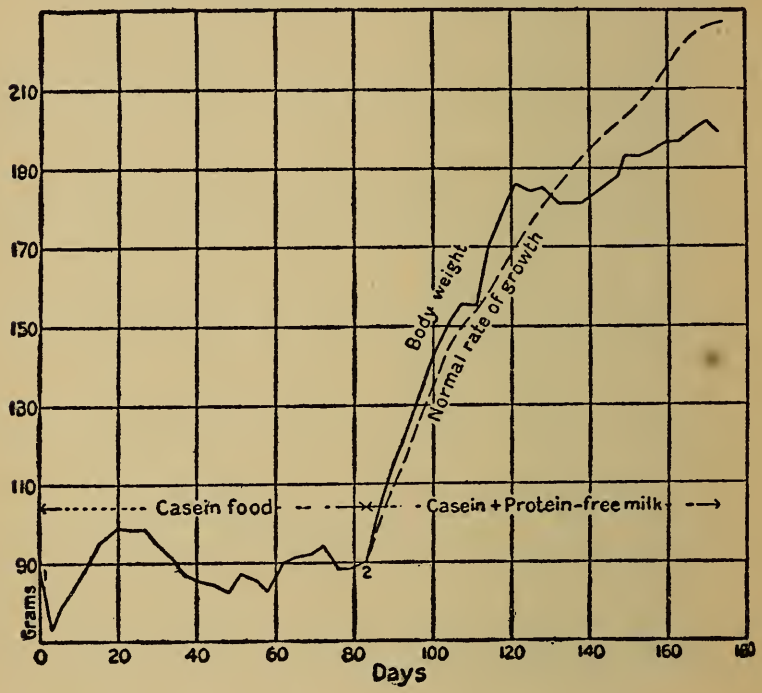


FIGURE 5. MAINTENANCE ON CASEIN AND GROWTH AFTER ADDITION OF PROTEIN-FREE MILK.

are supplied, such as are furnished by "protein-free milk" maintenance and growth in white rats may be normal. Emphasis should therefore be laid upon the importance of the rôle played by the accessory food-stuffs, as contained in "protein-free milk" the nature

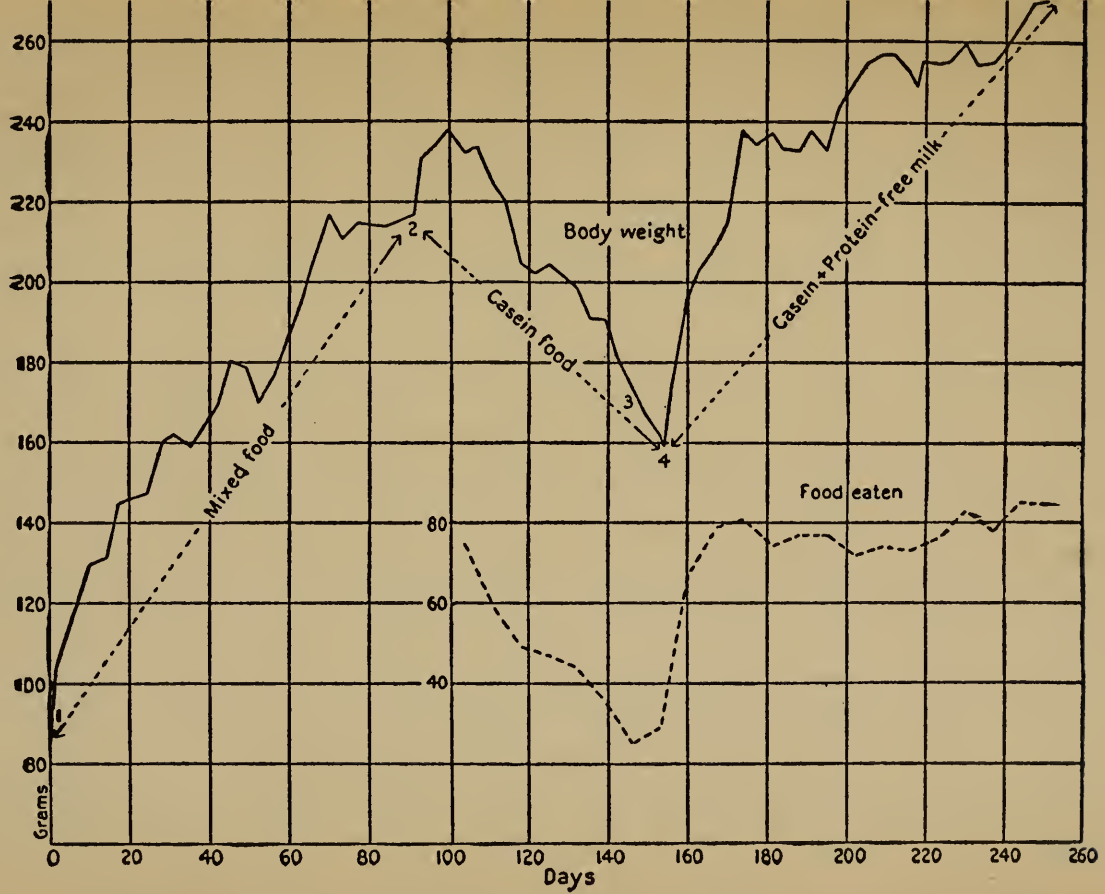


FIGURE 6. RESUMPTION OF GROWTH AFTER ADDITION OF PROTEIN-FREE MILK TO CASEIN DIET.

of which remains obscure. It is also evident that the establishment of a satisfactory non-protein dietary affords an opportunity for the study of any specific influence which a peculiar type of protein, or one with an unusual type of internal structure, may exert in nutrition.

In addition to casein Osborne and Mendel have demonstrated that perfectly satisfactory results may be yielded when other types of pure proteins are employed, a single one sufficing for all the nitrogen requirements of white rats. Thus, adequate growth has been secured with lactalbumin from cow's milk, ovalbumin from hen's egg, ovovitellin from hen's egg, edestin from hemp seed, cannabin from hemp seed, glutenin from wheat, glycinin from the soy bean, globulin from squash seed, globulin from cotton seed, excelsin from Brazil nut, and glutelin from maize.

Taking advantage of the opportunity afforded them, the above mentioned authors have studied the influence which a peculiar protein, for example, one lacking one or more important amino acid, may exert in nutritional processes. It soon became evident that all proteins do not promote growth under otherwise favorable conditions. Gliadins of rye and wheat, which are deficient in glycocoll and lysine and on the other hand are very rich in glutamic acid, and hordein of barley, which closely resembles gliadin in chemical constitution, are capable of giving maintenance, but fail to induce growth. A condition of stunting is brought about, old animals retaining the characteris-

tics of well-nourished young rats. In Fig. 7 are reproduced curves which show the failure of a rat to present normal growth on a diet containing protein-free milk and gliadin as the only protein. The

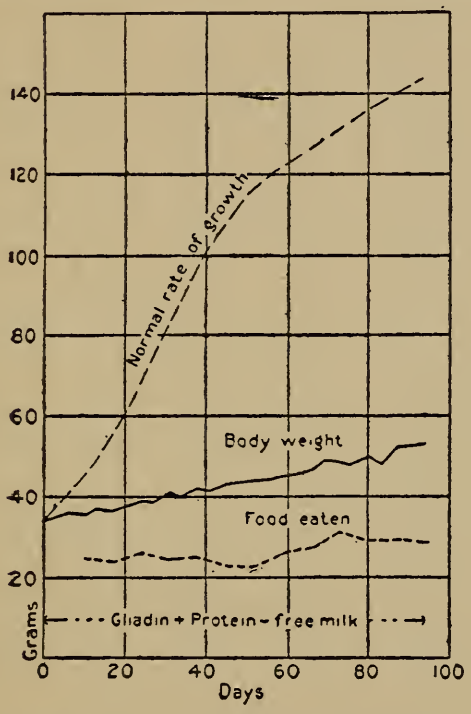
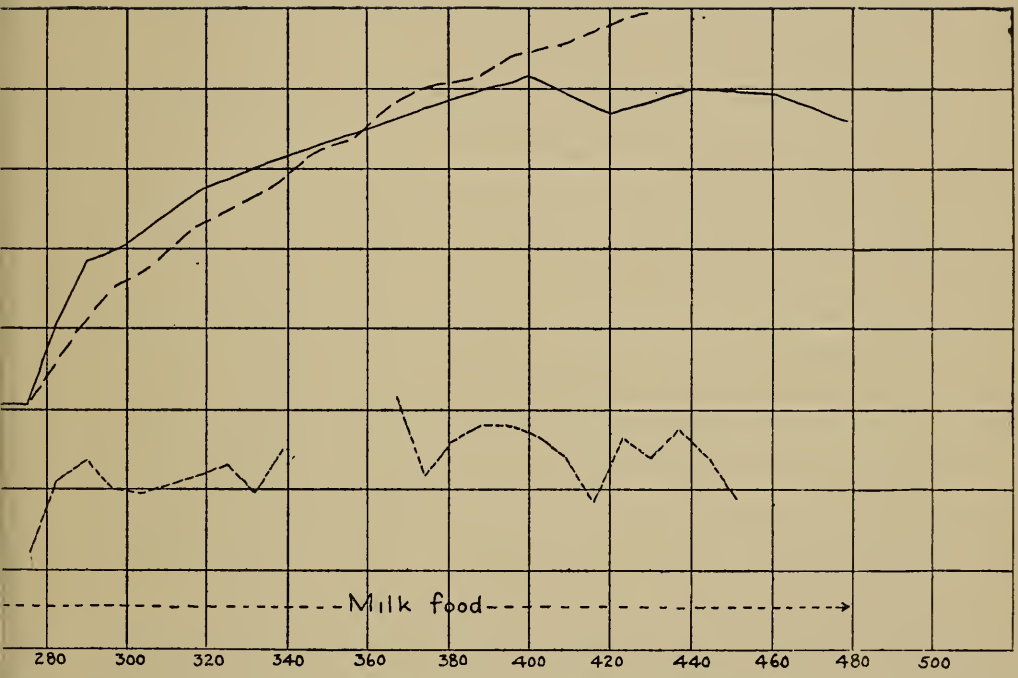


FIGURE 7. FAILURE OF GROWTH ON GLIADIN PLUS PROTEIN-FREE MILK.

frontispiece shows the photograph of this rat (B) and as a contrast that of a rat (A) of the same age presenting normal growth, together with a photograph of a rat (C) of the same weight as (B) but much younger. This stunting is apparently a method

which may be employed for the attainment of a type of animal infantilism. In connection with the subject of stunting it became of interest to determine whether this condition would remain permanent under all circumstances or whether a return to a diet containing a more typical protein than gliadin would also cause a resumption of growth. Fig. 8 shows the slight growth of a young white rat during 276 days of gliadin feeding. That the capacity to grow had not been lost, but was merely inhibited, may be seen in the second part of the curve in which milk food replaced the gliadin. At the beginning of the milk food diet the rat was 314 days old, an age at which rats usually show very little growth. Fertility is not impaired by the act of stunting, as may be seen from the curve in Fig. 9, for this rat, after a period of 154 days with gliadin as its protein supply, was mated and produced four young, which were suckled during the first month of their existence by the mother who was still maintained upon a gliadin diet. These young rats presented normal growth curves during this period. When a month old, three of the young animals were removed from the mother and kept upon diets of casein, edestin, and milk food. All showed normal curves of growth. The fourth young rat, kept with the mother began to exhibit a failure to grow as soon as forced to depend upon the gliadin food mixture. Inasmuch as casein, which has been proved to be efficient as a source of nitrogen for both maintenance and growth, is lacking in glycocoll,

FIGURE 8. This figure shows failure of rat to make more than slight gr
at a normal rate after 276 days of stunting. At this time the rat was 314 day
Biological Chemistry, volume 12.]



on a diet containing gliadin as the sole protein, and capacity to resume growth an age at which rats normally grow very little more. [From the *Journal of*

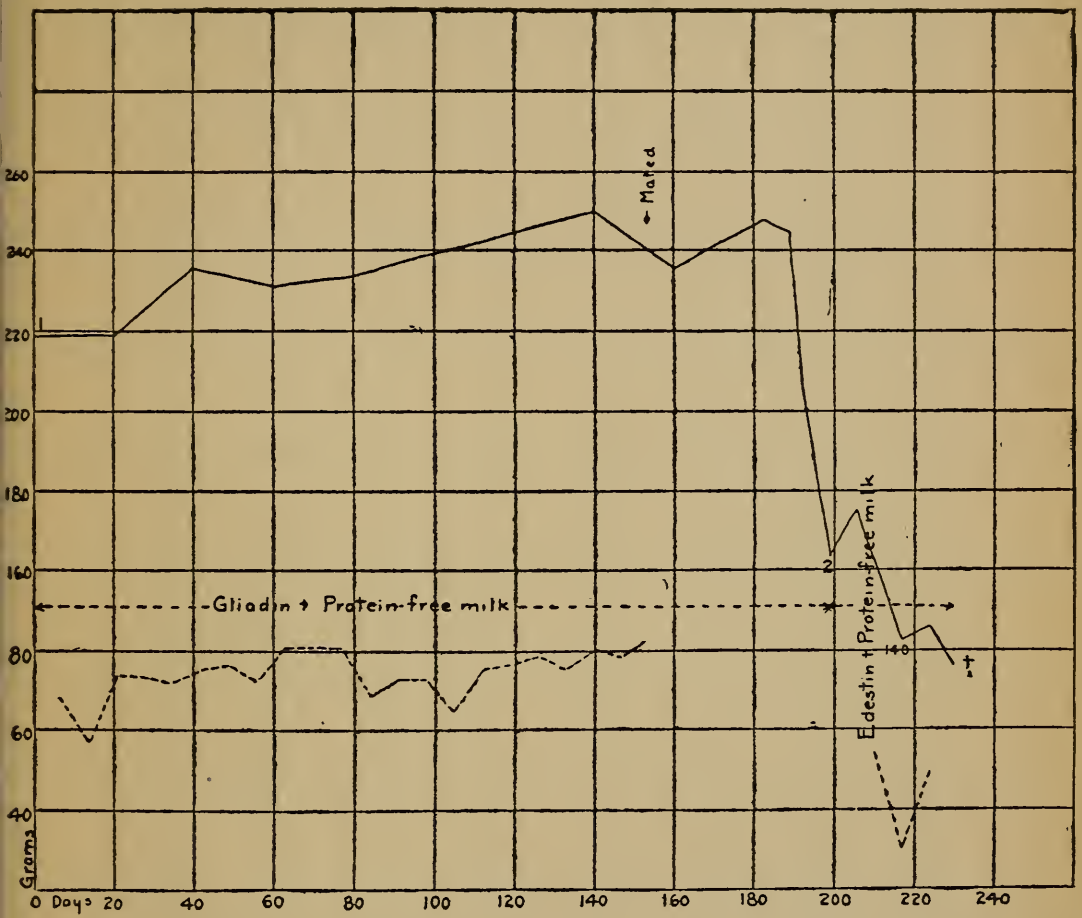


FIGURE 9 shows maintenance and fertility on a diet containing gliadin as its sole protein. After 154 days this rat was paired, four young being the result of the mating. [From the *Journal of Biological Chemistry*, volume 12.]

whereas gliadin is deficient in glycocoll and lysine and fails to promote growth, it is reasonable to assume that the low content of lysine in gliadin is responsible for the failure of white rats to grow. On the other hand, lysine is apparently not essential for mere maintenance. Another conclusion which may be drawn from these experiments is that the organism is unable to synthesize lysine, although glycocoll may be synthesized with apparent ease, as has been shown in previous pages of this book. Growth means the formation of new tissues and in the absence of sufficient lysine the construction of new tissue does not occur readily, or at least proceed at the normal rate. The inference that lysine is concerned with the function of growth may be tested from another viewpoint. If the animals fed with gliadin, lacking in lysine, show a failure to grow the addition of lysine to gliadin should be followed by a resumption of normal growth. Such trials have been made by Osborne and Mendel and the results obtained are most strikingly seen in the following curves. [See Fig. 10.] Failure to grow on gliadin as the sole protein is first shown in the curves followed by a period of growth when lysine was added to the diet. The subsequent withdrawal of the lysine is followed in each instance by a cessation of growth. If lysine is added again growth is again resumed at a normal, to cease again when lysine is taken away. These results lead to the conclusion that lysine is indispensable for the functions of growth. Data collected by Osborne and Mendel

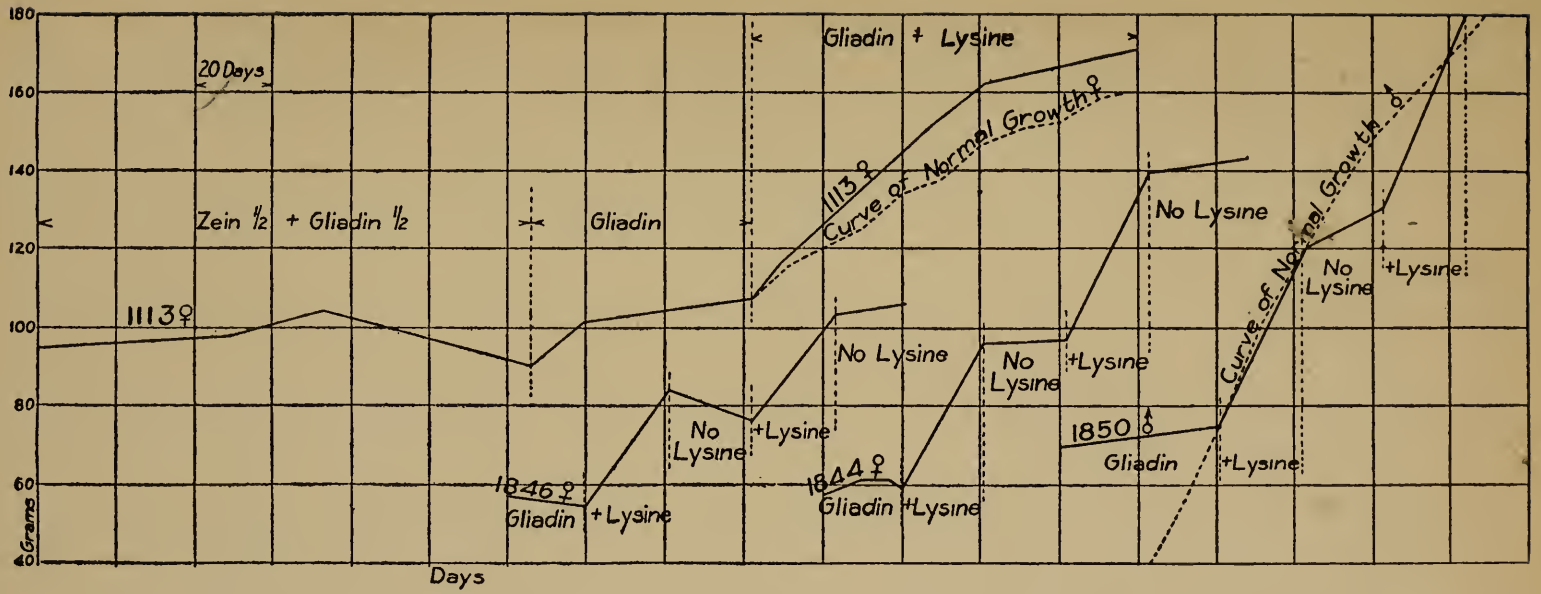


FIGURE 10. INDISPENSABILITY OF LYSINE FOR GROWTH. This chart shows the *failure to grow on diets containing gliadin* as the sole protein; the immediate resumption of *growth when lysine equivalent to 3 per cent of the protein is added to the food;* and the equally prompt *cessation of growth when the addition of lysine is stopped.* [From the *Journal of Biological Chemistry*, volume 17.]

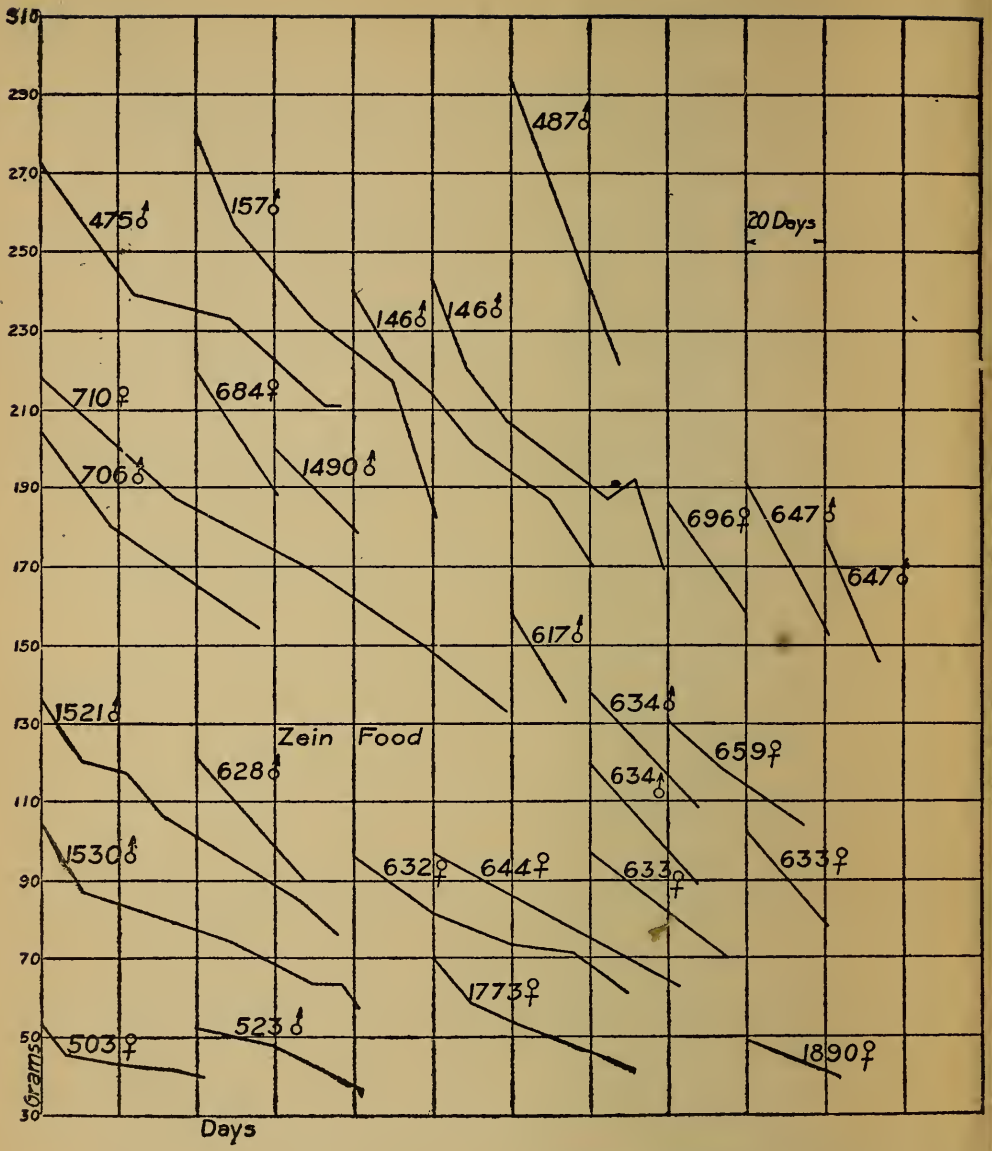


FIGURE 11. EXPERIMENTS WITH ZEIN. *Neither growth nor maintenance can be secured when zein is the sole protein in the dietary.* [From the *Journal of Biological Chemistry*, volume 17.]

reveal the "teleologically interesting fact . . . that those proteins, like casein, lactalbumin, and egg vitellin, which are in nature concerned with the growth of animals, all show a relatively high content of lysine."

The experience of these investigators with zein, which lacks glycocoll, tryptophane and lysine, has brought to light the fact that tryptophane is undoubtedly essential for maintenance and emphasizes anew the significance of lysine as a growth promoting substance. One may also assume that a little lysine is necessary for maintenance and this is ordinarily supplied in sufficient amount by the traces in gliadin or (in the zein and tryptophane experiments) by traces in protein-free milk protein or from the tissues themselves. In an earlier portion of this chapter were pointed out in some detail the experiments of Willcock and Hopkins with zein, with and without addition of tryptophane. They found that zein as the only protein in the dietary cannot maintain growth in the young animal nor even support life. The addition of tryptophane resulted in prolonging life without causing a resumption of the growth impulse. The outcome of the work of Osborne and Mendel with zein alone is best shown in the chart, Fig. 11. The large number of experiments shown here yielded concordant results and show that neither maintenance nor growth can be secured when zein is the only protein ingested. When tryptophane is added to the zein food mixture, maintenance of body weight follows, as may be seen from Fig. 12. Addition of both trypto-

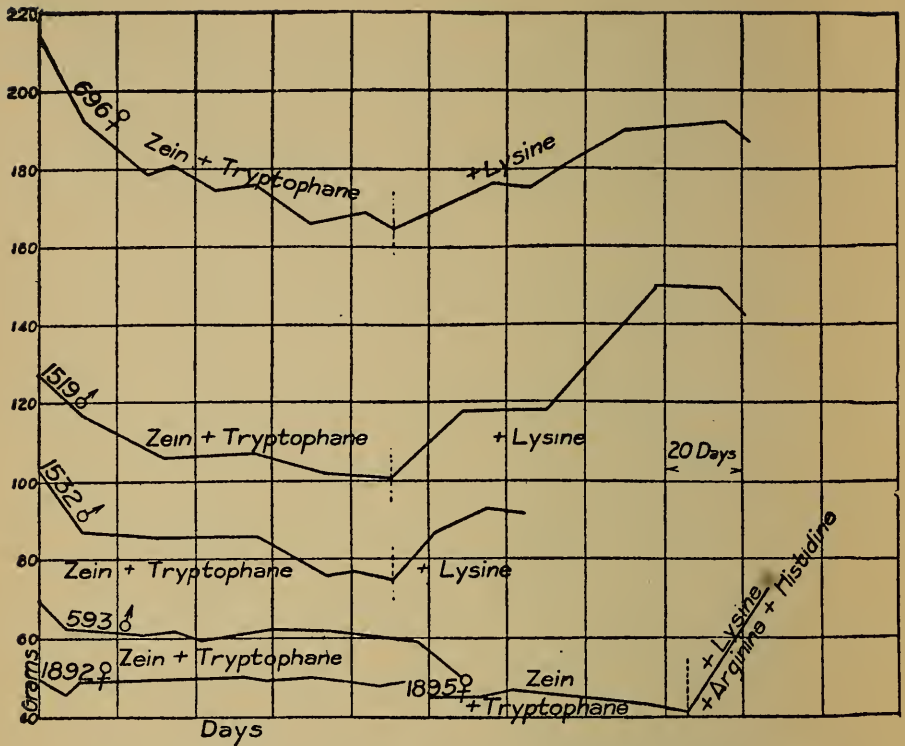


FIGURE 12. INDISPENSABILITY OF TRYPTOPHANE FOR MAINTENANCE IN NUTRITION. These experiments should be contrasted with the failure of maintenance on zein-food alone, shown in figure 11. [From the *Journal of Biological Chemistry*, volume 17.]

phane and lysine results in the establishment of perfect maintenance and growth. [See Fig. 13.] It may be inferred from these experiments then that tryptophane is indispensable for maintenance in nutrition and that the animal organism does not possess the ability to synthesize this amino acid. That lysine cannot replace tryptophane in the establishment of the condition of

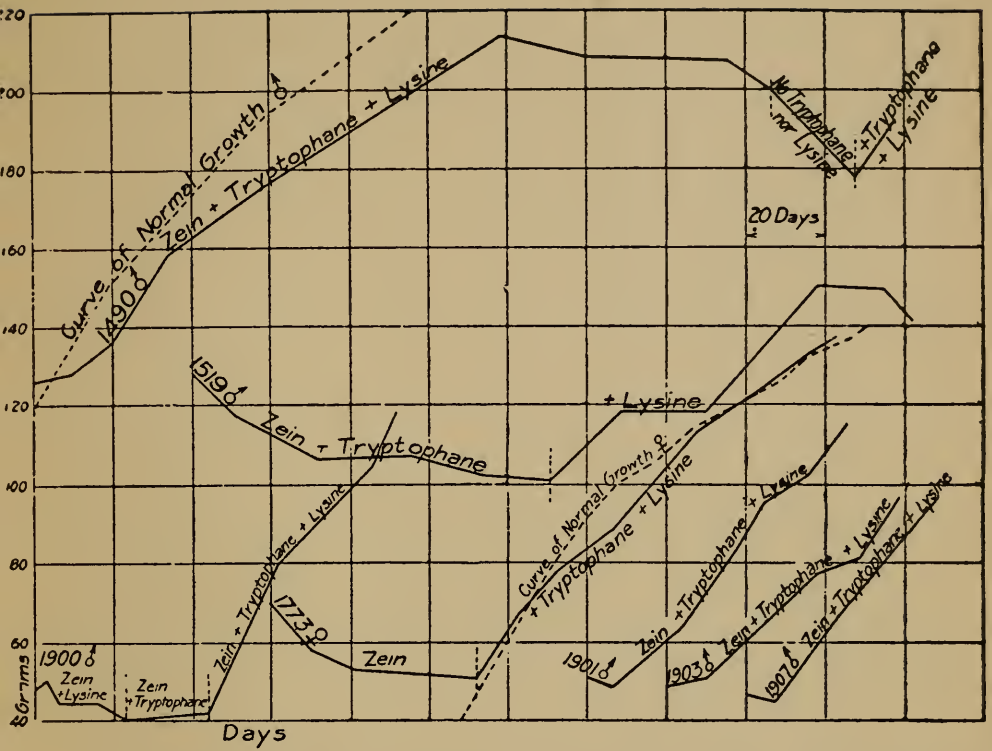


FIGURE 13. GROWTH ON FOODS CONTAINING ZEIN+TRYPTOPHANE +LYSINE. The *growth* obtained on this diet may be contrasted with *maintenance without growth* in the absence of the lysine (see Figure 12) and *failure to be maintained* in the absence of both lysine and tryptophane (Figure 11), thus demonstrating the rôle of these amino acids in growth and maintenance respectively. That *lysine cannot replace tryptophane in maintenance* is shown by Rat 1900. [From the *Journal of Biological Chemistry*, volume 17.]

maintenance, may be seen from the chart, Fig. 13. Rat 1900.

Investigation of this type into the biochemical deportment of the protein cleavage products will undoubtedly lead ultimately to the assignment of more or less specific functions to the various amino acids, and hence will indirectly indicate the relative efficiency of this or that protein in bringing about a desired result in nutrition.

References to Literature

Mendel: Nutrition and Growth: Journal of the American Medical Association. 1915, *64*, p. 1539.

Osborne and Mendel: Journal Biological Chemistry. 1914, *17*, p. 325.

Willcock and Hopkins: Journal of Physiology. 1906, *35*, p. 88.

INDEX

INDEX

ERRATA

Page 6, line 21. For Protomines, read Protamines.

Page 14, line 5. For *Isoleucine*. α-amino-β-ethyl-propionic acid, read *Isoleucine*. α amino methyl-ethyl propionic acid.

Page 14, line 25. For $HO.C_6H_5.CH_2.CH<^{NH_2}_{COOH}$,

read $HO.C_6H_4.CH_2.CH<^{NH_2}_{COOH}$

Page 16, line 13. For

$$\begin{array}{l} CH_2 \\ | \\ CH_2.COOH \\ | \\ CH.NH_2.COOH \end{array}$$

read

$$\begin{array}{l} CH_2.COOH \\ | \\ CH_2 \\ | \\ CH.NH_2.COOH \end{array}$$

Page 33, line 19. For 1906, read 1901.

Page 44, line 10. For

$$\begin{array}{l} CH_2.CH_2.CH_2.CH.COOH \\ | \qquad\qquad\quad | \\ NH \qquad\qquad NH_2 \\ | \\ C<^{NH_2}_{NH_2} \end{array}$$

Arginine

read

$$\begin{array}{l} CH_2.CH_2.CH_2.CH.COOH \\ | \qquad\qquad\quad | \\ NH \qquad\qquad NH_2 \\ | \\ C<^{NH}_{NH_2} \end{array}$$

Arginine

Page 111, lines 16 and 24 } For $C_6H_6O_6$, read $C_6H_{12}O_6$.
Page 112, lines 7 and 18 }

Page 119, line 6. For Ergebnisse des Physiologie, read Ergebnisse der Physiologie.

Page 121, line 12. For were metabolism, read were metabolized.